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LACTATE DEHYDROGENASE
AND ITS ISOENZYMES
IN TESTICULAR GERM CELL TUMORS

by
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The thesis is based on the following papers:

1. von Eyben, F.E. (1978): Biochemical markers in advanced testicular germ cell tumors. Serum lactate dehydrogenase, urinary chorionic gonadotropin and total urinary estrogens. *Cancer* 41,648-652.
2. von Eyben, F.E., Skude, G., Fosså, S.D., Klepp, O. and Bjørner, O. (1983): Serum lactate dehydrogenase (S-LDH) and S-LDH isoenzymes in patients with testicular germ cell tumors. *Molecular and General Genetics* 189, 326-333.
3. von Eyben, F.E., Jacobsen, G.K., Pedersen, H., Jacobsen, M., Clausen, P. P., Zibrandtsen, P.C. and Gullberg, B. (1982): Multivariate analysis of risk factors in patients with metastatic testicular germ cell tumors treated with vinblastine and bleomycin. *Invasion and Metastasis* 2, 125-135.
4. von Eyben, F.E., Skude, G. and Krabbe, S. (1982): Serum lactate dehydrogenase and its isoenzymes in men with maldescended testes. *Journal of Urology* 128,1195-1197.
5. von Eyben, F.E., Skude, G., Tropé, C., Wennerberg, J. and Mikulowski, P. (1982): Lactate dehydrogenase isoenzyme 1 (LDH-1) in athymic mice with xenografts of a human testicular germ cell tumor. *Molecular and General Genetics* 168,427-431.

LACTATE DEHYDROGENASE AND ITS ISOENZYMES IN TESTICULAR GERM CELL TUMORS: AN OVERVIEW

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Serum lactate dehydrogenase (S-LDH) was raised in 41 of 82 patients with testicular germ cell tumors, none of three with subclinical testicular tumors, and 10 of 341 without testicular germ cell tumors. 215 of whom had a previous history of tumor. 13 of 38 with testicular germ cell tumors, none of three with subclinical testicular germ cell tumors, and seven of 341 without tumor lesions had raised levels of isoenzyme S-LDH-1. 46 of 52 had concordant changes in S-LDH and total tumor volume whereas in six this relationship was not noted. Concordant changes in S-LDH-1 and total tumor volume were found in most cases. Higher S-LDH and S-LDH-1 levels were noted in the blood from the testicular vein on the side of the tumor at orchiectomy than in peripheral blood. The S-LDH activity predicted survival in a multivariate analysis of risk factors in 39 patients with stage 3 metastases from testicular germ cell tumors. 70-75% of 37 with tumor lesions had concordantly raised or normal S-LDH and serum concentrations of alpha-fetoprotein (S-AFP) and human chorionic gonadotropin (S-hCG); 25-30% had discordant levels. The diagnostic specificity of S-LDH and S-LDH-1 with regard to tumors in 252 with a history of testicular germ cell tumors was 68%, the diagnostic sensitivity 90%, the nosographic specificity 97%, the nosographic sensitivity 41% for S-LDH and 35% for S-LDH-1, and the effectiveness of the tests 88% for S-LDH and 90% for S-LDH-1. Tumor tissue from a testicular germ cell tumor transplanted into athymic mice had a high activity of LDH-1. There was a good correlation between S-LDH-1 and tumor volume. S-LDH may be used as a tumor marker in patients with testicular germ cell tumors in addition to other diagnostic tests. In patients with a history of testicular germ cell tumors and an unexplained elevation of S-LDH, raised S-LDH-1 may indicate the presence, and normal S-LDH-1 the absence, of testicular germ cell tumors.

Lactate dehydrogenase

Nude mice

Lactate dehydrogenase isoenzyme

Testicular neoplasm

INTRODUCTION

Testicular germ cell tumors

Incidence. Although a relatively rare disease, testicular germ cell tumors are the commonest forms of cancer in the Western world in 20-35-year-old men [1]. The incidence has increased recently [2,3]. For example, in Denmark, with the highest incidence [4], there were about three cases per 100,000 men in 1943. Now it is about nine per 100,000 men. The incidence, however, is lower in Sweden (three cases per 100,000 men in 1978) [5].

Histologic classifications. Testicular germ cell tumors are considered to arise from the germ cells of the testis [6]. Recent histologic classifications have been discussed in several reviews [7,8]. The classification of the American Force Institute of Pathology by Dixon and Moore [9] and others [10-13] and that of the Testicular Tumour Panel of Great Britain and Ireland [14,15] separate patients into different prognostic groups. The histologic patterns are often amassed into two main groups: seminomas and nonseminomatous tumors [16]. In about 90% of the patients, the histologic pattern of the metastases resembles that of the primary tumor [7,17].

Rarely do patients treated for testicular germ cell tumors develop nontesticular malignant tumors after orchiectomy [18,19].

Cellular origin. Various pieces of evidence support a germ cell origin for these tumors. Patients with atypical germ cells in the testicular tubules often subsequently develop invasive germ cell tumors [20,21]. Accordingly, atypical germ cells in the testis have been called carcinoma in situ [20]. The stage between carcinoma in situ and clinically overt tumors has been referred to as microinvasive [22].

Invasive testicular germ cell tumors may have seminomatous and/or nonseminomatous patterns. Thus, such tumors are an argument against the hypothesis of Willis [23] that nonseminomatous tumors do not originate from the germ cells while seminomas do.

Staging systems. Most staging systems divide the patients with testicular germ cell tumors into three main groups, according to their extent. In this review, the stage of primary tumor with or without extension to the spermatic cord is called stage 1: with metastases in only the retroperitoneal lymph nodes, stage 2: and with metastases in organs other than these lymph nodes, stage 3.

Before 1970, the survival rate of patients in stages 1, 2 or 3 differed markedly [2,13]. Recently, the overall survival has improved due to new chemotherapy regimes and improvements in monitoring the treatment of the disease [19,24]. Most patients with stages 1 or 2 disease now survive [25,26].

Tumor markers

General Aspects. Germ cell tumors may release a variety of molecules of assistance in staging, monitoring treatment, following-up after treatment, assessing prognosis, and assisting in classifying the histologic type of the tumor.

Ideally, for use as a tumor marker, a molecule should be released only by tumors. Most often, however, such molecules are released by both malignant and nonmalignant tissues. Nevertheless, if they are released to a greater extent from malignant tissue than from normal tissue, they may still have clinical utility.

Alphafetoprotein (AFP) and human chorionic gonadotropin (hCG) have been studied extensively [27,28]. Serum AFP (S-AFP) and serum hCG (S-hCG) are now

being determined routinely in patients with testicular germ cell tumors at diagnosis, in monitoring of treatment, and in follow-up. However, only half the patients have raised S-AFP or S-hCG levels at diagnosis [29]. Pregnancy-specific β_1 -glycoprotein (SP-1) [30,31] and placental alkaline phosphatase (Reagan isoenzyme, PLAP) [32-34] may also be clinically useful.

Other molecules which are not useful include serum carcinoembryonic antigen levels [32,35-37]. Serum α_1 -antitrypsin (S-AAT) was normal in seven patients with seminomas and only slightly raised in three of six with nonseminomatous tumors [38]. One-third of patients in two preliminary reports [40,41] had raised serum γ -glutamyl-transferase (S-GT, EC 2.3.2.2) [39]. These elevations might be due to liver disease. All patients in a small series with seminomas [42] or nonseminomatous tumors [43] had intermittently raised total urinary estrogens. In another series, however, only nine of 27 with tumor lesions had raised total urinary estrogens. The level did not predict the clinical course [44]. Wahren et al. [35] found that serum ferritin was raised in 50% of patients with tumors. However, Engelman et al. [45] claim that raised serum ferritin levels are due to anemia and not to tumor.

Lactate dehydrogenase (LDH)

Determination of LDH. The catalytic activity of LDH used to estimate its levels is measured by the conversion of its substrate (pyruvate or lactate). The Committee on Enzymes in Scandinavia recommend its determination by continuous measurement at 37°C and pH 7.4 with pyruvate as substrate. They also specify the equipment, and the calculation of the LDH activity from the measured results [46]. The International System of Units (the SI system) expresses LDH activity in kats (1 kat (katal) = 1 mol substrate converted by the enzyme per s) [47]. LDH activity may also be expressed in international units (1 I.U. = 1 μ mol substrate converted per min = 16.67 nkat) [39].

When determined according to the recommendations of the Commission on Enzymes in Scandinavia [46], the S-LDH activities vary less than those determined by earlier methods, and the interlaboratory precision is better [48,49]. The upper reference limits of S-LDH have been stated to be 7.5 μ kat/l (450 I.U.) [50], 8.0 μ kat/l (480 I.U./l) [51], or 8.4 μ kat/l (500 I.U./l) [52]; such discrepancies are unimportant.

Determination of LDH isoenzymes. LDH consists of five isoenzymes, numbered according to their electrophoretic mobility: LDH-1 has the highest and LDH-5 the lowest migration rate towards the anode [53]. Normal LDH isoenzymes are composed of four polypeptides (subunits) in different compositions of two subunits, denoted H and M: LDH-1 has four H subunits; LDH-2, three H and one M; LDH-3, two H and two M; LDH-4, one H and three M; and LDH-5, four M subunits.

LDH isoenzymes are often quantitated after they have been separated by electrophoresis and stained by formazan. The activities are determined accurately after electrophoresis in agarose gel [54] and may be quantitated using a densitometer

[55,56]. Estimated visually, LDH isoenzyme activities do not differ significantly from those measured densitometrically [57].

S-LDH-1 is determined slightly more accurately and precisely by electrophoresis than by immunoprecipitation, and much more accurately than by determination of urea-stable or heat-stable S-LDH or with the α -hydroxybutyrate test [58].

Several reviews deal with the methods for LDH isoenzyme determination [55,56,59,60]. So far, no method has been recommended by the international clinical chemistry societies.

LDH and LDH isoenzymes in normal men. All living cells have LDH in their cytoplasm. LDH is released into the blood both by leakage from living cells [61] and at cell death [50]. The range of normal S-LDH decreases with increasing age up to 22 years [61]. In older men the range is stationary. S-LDH at birth is twice the level in adults [62].

Normal man has five LDH isoenzymes. The fetus has a predominance of LDH-3 initially. During fetal development, this pattern changes into the different patterns of the adult [63]. In adults, heart, muscle and erythrocytes contain mainly LDH-1 and LDH-2; lung, prostate, thyroid, ovary and lymph nodes mainly LDH-2, LDH-3 and LDH-4; and liver, skeletal muscle and leukocytes mainly LDH-5. In normal adults, 30–40% of total S-LDH is LDH-1, 35–45% LDH-2, 20–30% LDH-3, about 3% LDH-4, and about 1% LDH-5 [50]. Postpubertal testes and sperm have an additional sixth LDH isoenzyme (LDH-X, LDH-C₄) [64]. Seldom are other additional isoenzymes found in man [65].

LDH and LDH isoenzymes in patients with non-neoplastic diseases. Patients with acute cell death (for example, myocardial infarction or pulmonary embolism) may have raised S-LDH. The elevation may be proportional to the mass of dead cells. S-LDH is maximally raised within 2–3 days after myocardial infarction. Later in the clinical course, S-LDH decreases at a rate of about 1% of the S-LDH per h until S-LDH is normal [66].

Patients with chronic diseases (for example, chronic hepatitis or muscular dystrophy) may have raised S-LDH for long periods.

The S-LDH isoenzyme pattern in patients with raised S-LDH reflects that of the diseased tissues. Patients with myocardial infarction or hemolytic anemia have mainly raised S-LDH-1 and S-LDH-2. Those with liver diseases (for example, hepatitis or cirrhosis) have mainly raised S-LDH-4 and S-LDH-5. Those with severe hypoxia may have a rise in all S-LDH isoenzymes.

LDH and LDH isoenzymes in patients with cancer, apart from testicular germ cell tumors. Patients with cancer often have raised S-LDH. Initial studies of S-LDH isoenzyme patterns in such patients have been reviewed [55,59,67]. Those with elevated S-LDH had mainly raised S-LDH-2, S-LDH-3 and S-LDH-4, or combinations of

these elevations, sometimes with raised S-LDH-5 in patients with liver metastases [68]. Women with dysgerminoma (a tumor with the same histologic pattern as seminoma) and elevated S-LDH had mainly raised S-LDH-1 [68].

These S-LDH isoenzyme patterns are very reproducible. Patients with cancer, apart from germ cell tumors, rarely have a dominant rise in S-LDH-1, a pattern noted in a patient with a mediastinal teratoma [69].

LDH AND LDH ISOENZYMES IN PATIENTS WITH TESTICULAR GERM CELL TUMORS

LDH and LDH-1 as tumor markers in testicular germ cell tumors

LDH and LDH-1 may act as tumor markers in a proportion of testicular germ cell tumors. Half the patients with testicular germ cell tumor metastases had raised S-LDH levels (Table 1) [37,44,70-80]. In contrast, only 3% of men without tumors in two series had raised S-LDH (three of 126 [79] and seven of 215 [80]). None of three patients with subclinical testicular germ cell neoplasms had raised S-LDH [79].

TABLE 1

S-LDH in patients with testicular germ cell tumors

Proportion of patients with seminoma who had raised S-LDH	Proportion of patients with nonseminomatous testicular germ tumors who had raised S-LDH	References
-	33/53	37
2/3	13/24	44 ^a
7/9	2/2	70
-	79/204	71
-	19/38	72
-	35/99	73
-	36/88	74 ^b
2/9	37/57	75
2/3	3/12	76 ^c
-	125/230	77
2/6	19/33	78
0/1	0/1	79 ^d
5/11	10/26	80
20/42	411/867	Totals

22 patients in Ref. 44 are included in the 39 patients of Ref. 78.

^a First determination of the study at Finseninstitutet.

^b Six patients evaluated both at initiation of treatment and after recurrence.

^c Four patients with stage I disease were not classified as to the histologic pattern of the primary tumor.

^d Two patients with carcinoma in situ are not classified as seminomas or nonseminomatous tumors.

In most series the raised S-LDH was due to anodal LDH isoenzymes. In one study, all patients with raised S-LDH had raised S-LDH-1 and S-LDH-2, while the other LDH isoenzymes were normal [70]. In another series 26 of 29 patients with raised S-LDH had raised S-LDH-1 without elevations of other LDH isoenzymes [71]. In a third series, 13 of 15 with raised S-LDH had raised S-LDH-1, often combined with elevation of S-LDH-2 and S-LDH-3 [80]. By contrast, only 2% of the men without tumors had raised S-LDH-1 (one of 126 [79] and six of 215 [80]). None of three patients with subclinical testicular germ cell neoplasms had raised S-LDH-1 [79]. In one series of 37 patients with testicular germ cell tumors, S-LDH-4 and S-LDH-5 were predominantly raised [81].

Concordant changes in S-LDH and tumor mass are usually noted in patients with testicular germ cell tumors [37,44,70,72,74,76,79,80,82,83]. In three series, 47 of 53 evaluable patients had concordant changes (Figs. 1-4) and six had discordant changes (Figs. 1 and 2) [44,79,80]. In a fourth study, 38 of 41 had concordant changes in S-LDH and other measures of disease apart from the tumor markers, and three had a rise in S-LDH with stable disease: one of the three had a rise in S-LDH during an episode of bacteremia and hypotension, and two had a rise in both S-LDH and S-hCG [37]. In a fifth study, all patients with stage 2 or 3 disease had concordant changes in S-LDH and tumor mass following initial treatment [72]. However, S-LDH levels normalized in three after orchiectomy before resection of retroperitoneal lymph node

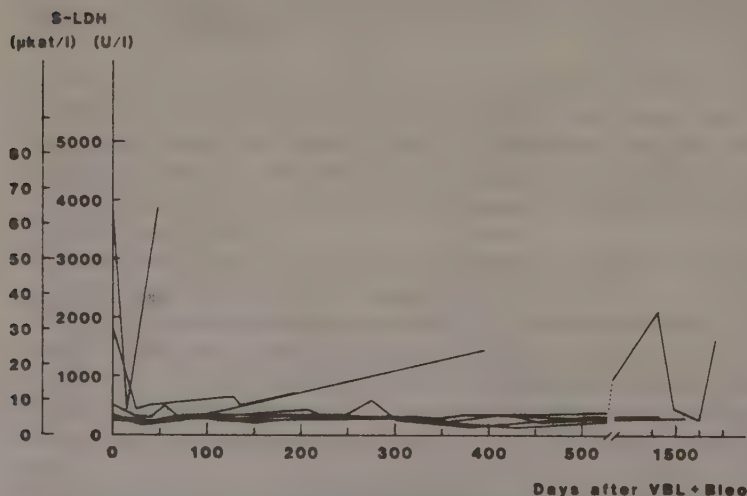


Fig. 1. Eight patients with metastatic germ cell tumors and complete remission following treatment with vinblastine and bleomycin (VBL + Bleo) [78]. All had a normalization of, or continued normal, S-LDH at the time of the remission, with a secondary rise in four who relapsed (one temporarily only) and in one with progressive pneumonitis.

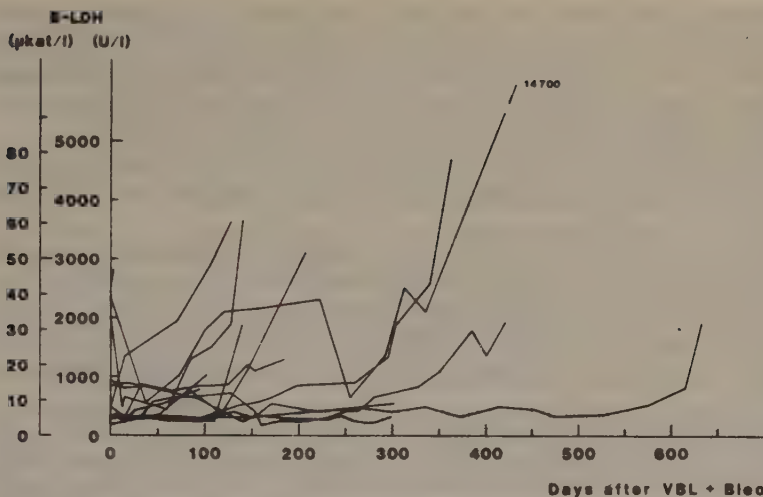


Fig. 2. 14 patients with metastatic testicular germ cell tumors and progression or treatment failure after vinblastine and bleomycin (VBL + Bleo) [78]. All but one had a rise in S-LDH with progression of the tumor.

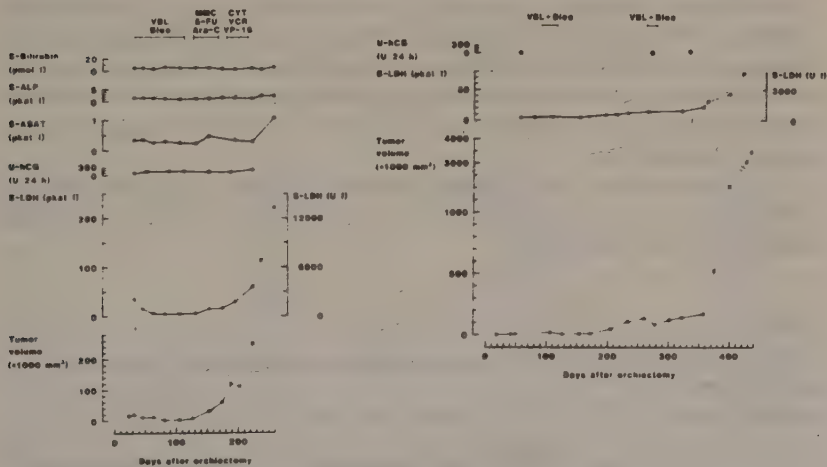


Fig. 3. A patient with initial remission and later progression [78]. S-LDH changed concordantly with tumor mass, while other liver function tests remained normal (vinblastine and bleomycin (VBL, Bleo); mitomycin C, vincristine and 1-arabinofuranosylecytosine, MMC, 5-FU, Ara-C, respectively; cyclophosphamide, vincristine, and etoposide, CYT, VCR, VP-16, respectively). S-ALP, serum alkaline phosphatase; S-ASAT, serum aspartate aminotransferase.

Fig. 4. A patient with metastatic testicular germ cell tumor and progression [78]. S-LDH and tumor mass changed concordantly without additional elevations during periods of treatment with vinblastine and bleomycin (VBL + Bleo).

metastases. Four patients had a recurrence, with normal S-LDH levels. In a sixth study, S-LDH and tumor mass changed concordantly in all patients [76]. S-LDH changed concordantly with the clinical course in 19 of 22 patients in another series [75]. Initial studies indicate that changes in S-LDH-I are less often discordant with those of the tumor mass compared with those of total S-LDH [76,80,84].

Blood from the testicular vein on the side of the tumor at orchiectomy has been shown to have higher S-LDH and S-LDH-I levels than arm vein blood [78,80] (Table II).

LDH-I was found to be the predominant isoenzyme in tumor tissue from a patient with seminoma [68,70] and from a patient with a testicular yolk sac tumor [85]. A high activity of LDH-I in tumor tissue was also found in another testicular germ cell tumor [86]. However, in one study, tumor tissue from patients with testicular germ cell tumors had predominantly raised cathodal LDH isoenzymes, LDH-4 and LDH-5 [81].

Some cells in a human testicular germ cell tumor transplanted to athymic mice were shown to have a high activity of LDH, by histochemical staining [86,87], or of LDH-I, by electrophoresis of tumor transplants extracts [86]. LDH-I has also been found in cyst fluid [86]. Similarly, tumor tissue from athymic mice with transplants of a testicular yolk sac had predominantly LDH-I [85]. S-LDH-I can also correlate with tumor volume in athymic mice with transplants of testicular germ cell tumors [85,86], probably due to the release of LDH-I into the blood from the tumor and not from non-malignant rodent tissues. Similarly, all cell lines from nonseminomatous tumors produce predominantly LDH-I [89]. In contrast, S-LDH-4 and S-LDH-5 increased concordant with tumor growth in another study with transplants of a nontesticular germ cell tumor with a predominance of LDH-4 and LDH-5 whereas S-LDH-I remained normal [88].

S-LDH was noted to correlate significantly with the tumor volume in several series [44,78,80] and with the tumor volume of a human testicular germ cell tumor transplanted to athymic mice [86].

TABLE II

S-LDH, S-LDH-I, S-AFP and S-hCG in testicular and systemic venous blood in a patient with a testicular germ cell tumor

	S-LDH (μ kat/l)	S-LDH-I ^a (μ kat/l)	S-AFP (μ g/l)	S-hCG (I.U./l)
Testicular vein blood	9.3	3.6	260	59
Cubital vein blood	4.2	1.3	170	20
Tumor cyst fluid	-	-	18 000	-

Upper limit of normal S-LDH-I, 3.0 μ kat/l.

Patients with stage 3 disease had raised S-LDH more often than those with stages 1 and 2 (Table III) [37,44,71-80]. The same proportion of patients with seminomatous and nonseminomatous tumor lesions have raised S-LDH (Table I).

Of 202 patients with nonseminomatous tumors, those with normal S-LDH (less than 225 I.U./l in the study) prior to treatment survived more than 8 years, those with S-LDH of 225-600 I.U./l 14 months, and those with S-LDH of more than 600 I.U./l, 10 months [71]. Similarly, the initial S-LDH had a significant prognostic impact in other patients with testicular germ cell tumor metastases (Fig. 5) [44,78,80]. Three of 11 patients with raised S-LDH and five of six with normal S-LDH were free of tumor at the time of the publication ($0.05 < P < 0.10$) [72] while 12 of 14 with raised S-LDH and one of eight with normal S-LDH after treatment died ($0.002 < P < 0.01$) [75]. In a multicenter trial of patients with stage 3 disease those with an initial S-LDH activity less than '3 times institutional norms' had a significantly higher rate of complete remission after chemotherapy with vinblastine, bleomycin and *cis*-platinum than those with higher initial S-LDH activities [90]. Patients with metastatic testicular germ cell tumors and S-LDH below 460 I.U./l have also been noted to fare better than those with higher S-LDH levels [73].

TABLE III

The proportion of patients with testicular germ cell tumors and raised S-LDH, according to stage

Stage 1	Stage 2	Stage 3	References
-	-	33/53	37
-	-	15/27	44 ^a
7/92	15/42	57/70	71
-	8/21	11/17	72 ^b
-	-	35/99	73 ^c
0/24	6/23	30/41	74 ^d
1/5	6/20	32/41	75
0/4	2/11	3/4	76
3/53	-	122/177	77 ^e
-	-	21/39	78
0/4	-	-	79
2/3	4/14	9/20	80
13/185	41/131	368/588	Totals

^a Staging and S-LDH at the start of the study at Finseninstitutet.

^b S-LDH before orchiectomy.

^c Stage 3 includes unresectable stage 2.

^d Stage 3 includes six patients with recurrence from stages 1 and 2 (the total number of patients is 82).

^e Stage 1 includes stage 2A (patients with total resection of retroperitoneal lymph nodes and normalization of S-AFP and S-hCG after the resection); stage 3 includes patients with subtotal or palliative resection of retroperitoneal lymph nodes.

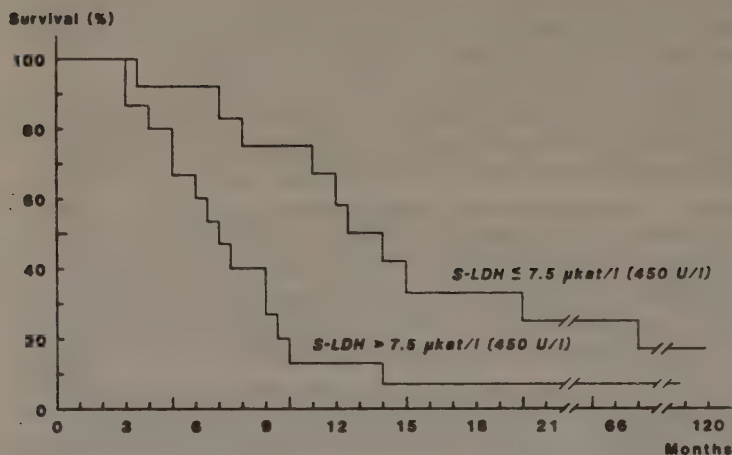


Fig. 5. 12 patients with metastatic testicular germ cell tumors and initial normal S-LDH levels survived significantly longer than 15 with initially raised S-LDH levels ($0.025 < P < 0.05$, log rank test) [44].

S-LDH and S-LDH-I did not prove useful to screen for testicular germ cell tumors in patients with a history of maldescended testes [79]. Similarly, S-LDH and S-LDH-I had no value in detecting microscopic testicular cancers [91]. One patients with a history of testicular germ cell tumor and with raised S-LDH had normal S-LDH-I and a nontesticular malignancy [80]. A similar case was reported in another series [92]. Electrophoretic S-LDH isoenzyme determinations in patients with testicular germ cell tumors did not show S-LDH-X [79,80]. Neither was it found in a study using an antibody against LDH-X [93].

Clinical value of S-LDH and S-LDH-I

Molecules used as tumor markers so far have only very limited value in detecting cancer in its initial localized phase [94]. Subclinical germ cell neoplasms were found only by testicular biopsy in a series of 130 men screened with various parameters [79,95,96].

At diagnosis, the level of S-LDH may separate patients with nonseminomatous testicular germ cell tumors into at least two different prognostic groups [71]. The value of including S-LDH and S-LDH isoenzyme determinations in staging is now being studied, especially in patients who do not undergo retroperitoneal lymph node resection as a staging and treatment procedure. S-AFP and S-hCG are known to increase the staging accuracy [97]. A normalization of raised S-AFP and S-hCG or both after orchiectomy according to the expected half-life is consistent with stage I tumors. If S-AFP or S-hCG or both are continuously raised after orchiectomy and

before the retroperitoneal lymph node are treated, metastases are present (stage 2 or 3), whereas constantly raised S-AFP or S-hCG after regional treatment implies stage 3 disease. Raised S-LDH and S-LDH-1, when not due to other diseases, may have similar implications.

S-LDH, S-LDH-1, S-AFP and S-hCG had the same efficacy with regard to tumor lesions in a series of 252 patients with a history of testicular germ cell tumor (Table IV) [80]. Combining S-AFP and S-hCG, or S-AFP, S-hCG and S-LDH-1 did not increase the effectiveness of the tests (Table IV). Nevertheless, both S-AFP and S-hCG are determined routinely in monitoring patients with testicular germ cell tumors as S-AFP and S-hCG may be discordant at diagnosis and in the clinical course. S-LDH or S-LDH-1 may also be useful as monitors, as they and S-AFP and S-hCG are discordant in one-third of patients (Table V) [37,72,74,75,80].

It is important to detect relapse early. Patients treated with chemotherapy due to raised S-AFP or S-hCG without other signs of metastases have a cure rate of 100% whereas patients with overt signs have a lower cure rate [19]. 44 patients with testicular germ cell tumors who had a recurrence in the follow-up after initial treatment had

TABLE IV

The diagnostic and nosographic values of the tests for measurable tumor lesions in 252 patients with a history of testicular germ cell tumors [80]

	S-LDH	S-LDH-1	S-AFP	S-hCG	S-AFP and S-hCG	S-LDH-1, S-AFP and S-hCG
Diagnostic specificity	0.68	0.68	0.79	0.71	1.00	0.90
Diagnostic sensitivity	0.90	0.90	0.91	0.91	0.89	0.90
Nosographic sensitivity	0.41	0.35	0.46	0.40	0.27	0.30
Nosographic specificity	0.97	0.97	0.98	0.98	1.00	0.99
Effectiveness of the test	0.89	0.88	0.90	0.89	0.90	0.90

The diagnostic specificity (the predictive value of a positive test) denotes the number of patients with neoplasm and a raised serum values out of all patients with raised test results [111]. The diagnostic sensitivity (the predictive value of a negative test) denotes the number of patients without neoplasm and with normal serum values out of the patients with a normal test [111]. The nosographic sensitivity ('sensitivity') denotes the number of patients with neoplasm and a raised test in patients with neoplasms [111]. The nosographic specificity ('specificity') denotes the number of patients without neoplasm and with a normal test in patients without neoplasm [111]. The effectiveness of the test denotes the number of patients with neoplasm and a raised test and the patients without neoplasm and with a normal test out of all tested patients [112]. As for the evaluation of both S-AFP and S-hCG, a positive test infers that both tests were positive, whereas for S-LDH-1, S-AFP and S-hCG together, a positive test was defined as two or three of the tests being positive.

TABLE V

S-LDH, S-AFP and S-hCG in patients with metastatic testicular germ cell tumors

S-LDH	S-AFP	S-hCG	Raised S-LDH, normal S-AFP and S-hCG	Raised S-AFP, normal S-LDH and S-hCG	Raised S-hCG, normal S-LDH and S-AFP	Raised S-LDH or S-AFP or S-hCG or combinations thereof	References
25/41	30/41	26/41	3/41	5/41	3/41	38/41	37
35/99	59/100	64/100	-	-	-	-	72
36/88	28/88	28/88	-	-	-	46/88	74
39/66	41/66	42/66	-	-	-	54/67	75
125/230	134/230	121/230	-	-	-	198/230	77
11/30	13/30	4/30	4/30	6/30	2/30	19/30	80
271/554	305/554	285/554	7/71	11/71	5/71	355/554	Totals

The table shows the proportion of patients with a raised S-LDH, S-AFP and S-hCG studied as single tests, as well as the proportion with a raised serum level in only one test, and the proportion with a raised serum level in one or more than one of the tests.

* Patients without determination of S-AFP or S-hCG or both at the time of the first S-LDH determination are not included in the Table.

had frequent determinations of S-LDH, S-AFP and S-hCG [77]. 22 had raised S-AFP or S-hCG or both before other signs of relapse, and five had raised S-LDH as the first sign of recurrence. S-AFP, S-hCG or S-LDH or combinations of these markers were raised concomitantly with other signs of recurrence in 11. Six subjects had normal S-AFP, S-hCG and S-LDH at the time of relapse, as detected by other investigations. So, in addition to S-AFP and S-hCG, S-LDH should be used to detect relapse.

S-LDH was the most important single factor to predict complete remission by chemotherapy in multivariate analyses of potential risk factors in 220 patients with metastatic testicular germ cell tumors treated at either the Memorial Sloan-Kettering Cancer Center or the University of Minnesota [113]. Similarly, S-LDH was a significant prognostic factor in another series [78].

Together with other patient characteristics indicating poor prognosis, for example, impaired performance status and large total tumor volume [78], S-LDH may be used to define a subgroup of patients with testicular germ cell tumor metastases who are seldom cured by the vinblastine, bleomycin and *cis*-platinum regimen [19]. Consequently, such poor-risk patients could be treated differently: e.g., increased doses of *cis*-platinum in combination with vinblastine and bleomycin [98], the addition of etoposide, or courses of vinblastine, bleomycin and *cis*-platinum alternating with etoposide and *cis*-platinum (together with doxorubicin and bleomycin) [99]. However, as an initial treatment in patients with metastatic testicular germ cell tumors at poor risk, cytoreductive surgery and chemotherapy did not prove better than chemotherapy alone [100].

In addition to clinical data, technical aspects of S-LDH determination support its use as a tumor marker in patients with testicular germ cell tumors. Determination of S-LDH is cheaper, quicker and more widely available than other diagnostic tests in routine use today for patients with testicular germ cell tumors [101].

Recommendations for the use of S-LDH and S-LDH-I

S-LDH and S-LDH-I should not be used as the only tests for screening for testicular germ cell neoplasms in a subclinical stage.

In patients with testicular germ cell tumors, S-LDH should be determined every month for the first year after diagnosis and every second month during the second year [27,28]. Later in the clinical course, S-LDH should be measured only as clinically indicated, as few patients relapse in this period [19].

S-LDH isoenzymes should be determined in patients with a history of testicular germ cell tumor and an unexplained elevation of S-LDH: raised S-LDH-I is a sign for, and a normal S-LDH-I a sign against, the presence of tumor.

Its measurement is also valuable in achieving a more accurate staging assessment and as a prognostic indicator.

Changes in LDH isoenzymes in the malignant transformation of testicular germ cells

Two explanations have been given for the elevations of S-LDH in patients with testicular germ cell tumors. Lieskovsky and Skinner [72] suggested that the elevations were caused by increased glycolysis and lactate production in the tumors due to hypoxia. However, it has not been demonstrated so far that the oxygen tension is lower in tumors of patients with raised S-LDH than in those of patients with normal S-LDH. Contrary to this suggestion, only some tumor cells had high activity of LDH by histochemical staining of a transplanted human testicular germ cell tumor [86]. They were not localized predominantly near necrotic areas of the tumor. Besides, the cathodal LDH isoenzymes might have been predominant [59], if hypoxia was the only or main cause for the release of LDH into the blood of patients with testicular germ cell tumors.

Javadpour [102] has suggested that the S-LDH elevations are due to increased glycolysis and lactate synthesis in the tumor compared to normal cells. However, tumors have different levels of anaerobic glycolysis: for example, the Morris hepatoma [103] or PCC4 embryonal carcinoma cell line [104]. Similarly different levels of S-LDH activity have been found in patients with tumors of the same histologic pattern (Table I).

The assumption that there is increased anaerobic glycolysis in testicular germ cell tumors accords with the experiments of Warburg [105], who found that tumor cells produce lactate in an oxygen-rich milieu while normal cells produce almost none. He claimed that tumor cells produce lactate ('glycolysis') in an oxygen-rich milieu as a

result of a defect in oxygen uptake by the cells. He claimed that cancer results when normal cells respond to an irreversible injury by acquiring anaerobic glycolysis. This interpretation of the experiments has been questioned [106]. Several clinical findings of S-LDH in patients with testicular germ cell tumors do not fit with Warburg's hypothesis. If LDH was released from the tumors due to a primary and obligatory biochemical change on the malignant transformation of testicular germ cells, all patients with tumor lesions should have had elevations of S-LDH early in the clinical course. This is not the case (Table III).

Greenstein [107] suggested that similarities in growth of a malignant tumor and the embryo may explain the changes in enzymes in tumors: undifferentiated tumors might relate metabolically to early embryonic cells of the organ whereas the differentiated tumors might relate to late embryonic cells. Correspondingly, some experimental and human tumors have shown the isoenzyme pattern of embryonic cells [108]. At present, it is not clear whether the predominance of anodal LDH isoenzymes in some patients with testicular germ cell tumors reflects a fetal pattern typical of the normal testis [109].

Weber [110] classes the enzymatic changes on malignant transformation into three groups: 1, those that correlate with the degree of malignancy defined as measured growth of various tumor lines (progression-linked changes); 2, those found in all tumors of a certain type (transformation-linked changes); and 3, coincidental changes that do not relate to malignancy [110]. He suggested that neither transformation- nor progression-linked LDH changes in neoplasms [110]. But the changes in LDH isoenzymes in malignant transformation of testicular germ cells may be both transformation- and progression-linked. All normal adult testicular tissues have LDH-X and other additional LDH isoenzymes [64], whereas these isoenzymes are not found in testicular germ cell tumors [68,85,86]. LDH-1 is the predominant LDH isoenzyme in tumor tissue and serum in some patients with testicular germ cell tumors, but not in normal testicular germ cell tissue [64].

CONCLUSIONS

LDH and LDH-1 satisfy five criteria as tumor markers in a proportion of patients with testicular germ cell tumors. First, half the patients with testicular germ cell tumors have raised S-LDH and one-third raised S-LDH-1 levels. Second, most patients with raised S-LDH and tumors have concordant changes in S-LDH and tumor mass and nearly all have concordant changes in S-LDH-1 and tumor mass. Third, higher activities of S-LDH and S-LDH-1 are found in the blood from the ipsilateral testicular vein than in peripheral venous blood. Fourth, high activities of LDH-1 can be found in testicular germ cell tumor tissue. Lastly, some cells in a human testicular germ cell tumor transplanted to athymic mice had high LDH activity, as assessed by histochemical staining for LDH. Further analysis showed that the high activity was due to LDH-1.

S-LDH may be used together with other patient characteristics as a prognostic index. The enzyme levels should be determined every month for the first year after diagnosis and every second month during the second year. S-LDH isoenzyme determinations should be undertaken in patients with a history of testicular germ cell tumor and an unexplained elevation of S-LDH.

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BIOCHEMICAL MARKERS IN ADVANCED TESTICULAR TUMORS

Serum Lactate Dehydrogenase, Urinary Chorionic Gonadotropin and Total Urinary Estrogens

FINN EDLER VON EYBEN, MD

Twenty-seven patients with advanced testicular tumors were screened for serum lactate dehydrogenase (LDH) concentration, and 24-hour urinary excretion of human chorionic gonadotropin (hCG) and total estrogens. The investigation indicates an overall correlation between serum LDH concentration and cancer burden and an overall correlation between the maximum serum LDH and prognosis. It indicates an overall correlation between hCG determined within 2.5 months after the orchiectomy and prognosis. The relation between total estrogens and cancer burden or prognosis does not support the hypothesis that total urinary estrogens are a biochemical marker in testicular tumors.

Cancer 41:648-652, 1978.

BRINDLEY AND FRANCIS¹ FOUND A STATISTICALLY significant correlation between changes in serum lactate dehydrogenase (LDH) concentration and cancer burden. Two patients with testicular carcinoma had elevated LDH before treatment and a positive correlation between LDH level and tumor change during treatment. Urinary chorionic gonadotropin (hCG) determination has been reported to be of prognostic value in testicular tumors,^{4,5} especially during the postoperative period.

Lindenmeyer *et al.*^{7,8} reported a relation between estrogen excretion and the presence of malignant tissue in three out of 12 patients with testicular tumors. Four out of seven patients with total urinary estrogens elevated to 40 $\mu\text{g}/24$ h died during the observation period or more versus one out of five patients with estrogens less than 40 $\mu\text{g}/24$ h.

The purpose of this report is to correlate serum LDH, urinary hCG and total urinary estrogens with an estimation of the actual cancer burden in advanced testicular tumors and to reexamine the prognostic value of these biochemical labels.

MATERIALS AND METHODS

Patients

The biochemical labels were determined in 27

patients with advanced testicular tumors admitted to Finseninstitutet between August 1972 and February 1975 to receive chemotherapy.

The primary tumor was histologically verified in all patients and classified according to the criteria of Dixon and Moore.⁴ The stage was determined according to the criteria of Boden and Gibb¹ (Table 1). Advanced tumor was histologically verified in 22 patients. When orchiectomy took place, the median age was 28 years with a range from 16 to 52. None of the patients had had myocardial infarction, and none were treated with digoxin. None of the patients were dysthyroid. Four patients had a slightly increased serum creatinine.

Treatment

In all cases, the initial treatment was orchiectomy. At stages 1 and 2, all patients received postoperative radiation of the paraaortic and pelvic lymph nodes. Advanced tumors were treated with radiation before chemotherapy, a combination of radiation and chemotherapy, or chemotherapy. Twenty-four patients received combination chemotherapy with bleomycin twice weekly and vinblastine with a five-week interval for a maximum of 10 weeks. Patients with complete remission were observed without longterm maintenance therapy. Patients with persisting or progressing cancer lesions received other chemotherapy regimens until they were considered terminal.

Laboratory Analyses

Serum LDH concentration was measured by the method of Wroblewski and LaDue¹⁹ modi-

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TABLE 1. Distribution of Patients by Pathologic Type and Clinical Stage

Clinical Stage	No. of cases	Seminoma	Embryonal carcinoma with or without seminoma	Teratoma with or without seminoma	Teratoma with either embryonal carcinoma or choriocarcinoma or both with or without seminoma	Choriocarcinoma with either embryonal carcinoma or seminoma or both
I	8	1	3	3	1	0
II	7	2	4	1	0	0
III	12	0	7	1	3	1
TOTAL	27	3	14	5	4	1

Stage I is local tumors confined to testis and adnexa. Stage II is tumors involving regional lymph nodes. Stage III is tumors involving other areas than regional lymph nodes (advanced tumors).

fied by KABI. LDH was determined when chemotherapy was initiated and at scheduled intervals during the treatment. Four to 26 determinations with a median of 11 were made.

The patients collected 24-hour samples of urine in plastic containers, without using preservatives. Before 1972, urinary hCG was determined by the biologic method of Hamburger and Pedersen-Bjergaard³ in three samples. From 1972 on, the hCG content of the samples has been measured by the radioimmunologic method of Midgley¹⁰ with a few modifications. In the case of values less than 300 IU/24 h, the method either shows hCG, luteinizing hormone or a mixture of both. At the beginning of the postoperative treatment, urinary hCG was determined in 24 patients. Two to nine determinations with a median of four were made.

The total urinary estrogen content was measured by the fluorimetric method of Brown *et al.*,⁸ modified by Fahmy and Frandsen.⁶ In 12 patients, total urinary estrogens were determined when treatment with bleomycin and vinblastine was started. One to six determinations of total urinary estrogens with a median of two were made.

Estimation of Cancer Burden

In each cancer lesion measurable by physical examination, chest roentgenogram and lymphogram, the tumor area was calculated as the product of the greatest length and breadth. The sum of the tumor areas in each patient at a given time was used as an estimation of the total cancer burden.

Control Group

A 24-hour urinary excretion of total estrogens was determined in 18 healthy men where the median age was 27.5 years and the range from 15 to 50 years.

RESULTS

Total Urinary Estrogens Excretion in Patients with Testicular Tumors and in Normal Men

The median of the mean values of total urinary estrogens in patients with testicular tumors was 20.5 $\mu\text{g}/24\text{ h}$ and the range from 5 to 53.5 $\mu\text{g}/24\text{ h}$. The median value of the control group was 13 $\mu\text{g}/24\text{ h}$ and the range from 7 to 30 $\mu\text{g}/24\text{ h}$. There was a statistically significant difference between the mean values of the cancer patients and the control group ($p = 0.03$, Mann-Whitney U test, two-tailed).

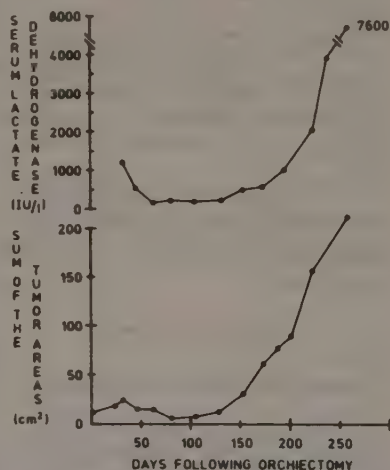


FIG. 1. Serial LDH values in a case with a temporary regression of cancer burden. Normal LDH is below 200 IU/l.

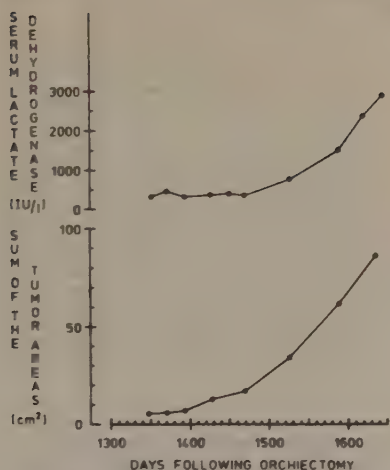


FIG. 2. Serial LDH values in a case with a progressing cancer burden.

Serum LDH Concentration, Urinary hCG and Estrogen Excretion, and Cancer Burden

The total urinary estrogens of nine patients were elevated (i.e., more than 30 $\mu\text{g}/24\text{ h}$), whereas

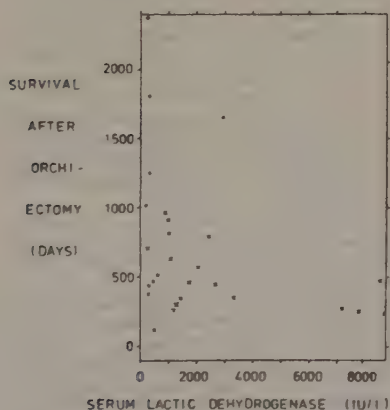


FIG. 3. Relation between survival and maximum serum LDH. Crosses represent the patients who died during the study. Dots represent the patients still alive. There were 27 patients. The spearman rank correlation coefficient r_s was -0.4739 ($0.01 < p < 0.02$, two-tailed test).

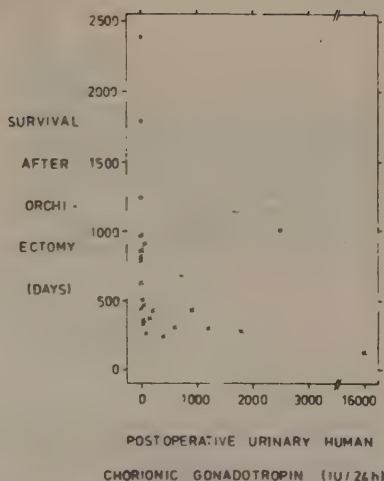


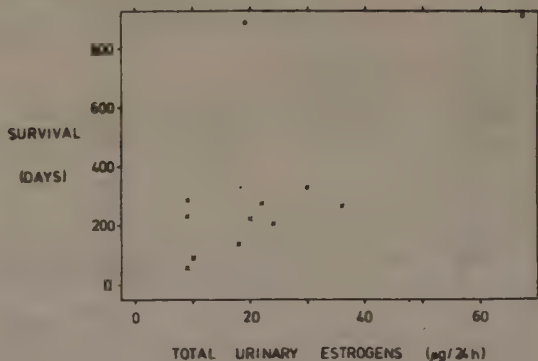
FIG. 4. Relation between survival and urinary hCG determined within 2.5 months after the orchiectomy. There were 24 patients. Crosses represent the patients who died during the study. Dots represent the patients still alive. The Spearman rank correlation coefficient r_s was -0.6286 ($0.001 < p < 0.01$, two-tailed test).

13 patients had urinary hCG excretion (i.e., hCG values of more than 300 IU/24 h), and 27 patients had an elevated serum LDH maximum (i.e., 200 IU/l or more). The three hCG determinations by the biologic method were negative. There was an overall correlation between LDH and cancer burden ($p < 0.001$, Spearman rank correlation coefficient test, two-tailed). Figure 1 shows the time-course for LDH in a patient whose cancer burden was reduced and Fig. 2 the time-course in a patient whose cancer burden progressed. There was no overall correlation between estrogen or hCG excretion higher than 300 IU/24 h and cancer burden ($p > 0.2$, Spearman rank correlation coefficient test, two-tailed).

Serum LDH Concentration, Urinary hCG and Estrogen Excretion, Clinical Stage and Prognosis

There was an overall correlation between the maximum serum LDH concentration and prognosis ($0.01 < p < 0.02$, Spearman rank correlation coefficient test, two-tailed) (Fig. 3). Four

FIG. 5. Relation between total urinary estrogens and survival after the determination when treatment with bleomycin and vinblastine was started. Crosses represent the patients who died during the study. Dots represent the patients still alive. The Spearman rank correlation coefficient r_s was 0.3829 ($p > 0.2$, two-tailed test).



patients with a serum LDH maximum of more than 5000 IU/l had a poorer prognosis than 23 patients with less than 5000 IU/l ($0.001 < p < 0.01$, logrank test, two-tailed).

There was an overall correlation between postoperative hCG and prognosis ($0.001 < p < 0.01$, Spearman rank correlation coefficient test, two-tailed) (Fig. 4). Figure 5 shows the relation between total urinary estrogens determined when treatment with bleomycin and vinblastine was started and the survival after the determination. There was no overall correlation between total urinary estrogens and survival ($p > 0.2$, Spearman rank correlation coefficient test, two-tailed). When using the staging system of Boden and Gibb,¹ a correlation between the stage of the disease and survival was found ($0.01 < p < 0.025$, Spearman rank correlation coefficient test, two-tailed).

DISCUSSION

The reason biochemical labels are of interest in cancer treatment is a supposition that specific tumor products can be used as markers on which to base diagnosis, monitor therapy, perform follow-up studies, ensure remission and estimate prognosis. This study correlates an enzyme and two hormones previously reported as biochemical markers in testicular tumors with cancer burden and prognosis in advanced testicular tumors.

Like previous studies,^{4,9} this investigation indicates that a post-orchietomy determination of urinary hCG is of prognostic value. Never-

theless, we did not find an overall correlation between cancer burden and urinary hCG determination higher than 300 IU/24 h. This discrepancy may be due to different mixtures of hCG-secreting and non-hCG-secreting elements of testicular tumors, or to a different sensitivity to the treatment of these elements, or both.

Like the reports of Lindenmeyer *et al.*,^{7,8} this study shows that elevated values of total urinary estrogens can be determined in testicular tumors. When treatment with bleomycin and vinblastine was started, higher values tended to occur in patients who lived longer. Therefore this investigation does not support the hypothesis that total urinary estrogens is a biochemical marker of testicular tumors. Furthermore, we did not find a correlation between cancer burden and total urinary estrogens. Symington and Wallace¹² found no difference between the output of oestriol, oestrone and oestradiol in patients with testicular tumors and normal men, but no details were reported.

This study shows a correlation between LDH and cancer burden, and between maximum LDH determination and the prognosis in advanced testicular tumors. Human chorionic gonadotropin beta subunit and alpha-fetoprotein are more specific biochemical markers than LDH when diagnosing minimal cancer burden in testicular tumors because LDH levels below 200 IU/l occur in normal men and levels above 200 IU/l accompany many diseases besides testicular tumors. Prospective studies are necessary to evaluate whether LDH can be used in the treatment of testicular tumors.

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Serum Lactate Dehydrogenase (S-LDH) and S-LDH Isoenzymes in Patients with Testicular Germ Cell Tumors

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Summary. The activities of serum lactate dehydrogenase (S-LDH) and S-LDH isoenzymes were determined in 252 patients with a history of testicular germ cell tumors (TGCT). Fifteen of 37 patients with TGCT lesions and seven of 215 without had raised levels of S-LDH (above 8.0 $\mu\text{kat/l}$ (480 U/l)). Of the patients with TGCT lesions, four had only raised S-LDH-1 levels, one only raised S-LDH-2 (and normal S-LDH), two only raised S-LDH-3 (one with normal S-LDH), and 10 had five combinations of raised levels of S-LDH isoenzymes with a predominance of S-LDH-1. S-LDH and S-LDH-1 correlated significantly with the total tumor volume in the patients with TGCT lesions, especially pronounced in those with lesions from seminoma. Of 34 patients with TGCT metastases, 13 with raised S-LDH levels lived significantly shorter lengths of time than 21 with normal S-LDH. Similarly, 11 with raised S-LDH-1 (above 3.0 $\mu\text{kat/l}$ (180 U/l)) lived significantly shorter times than 23 with normal S-LDH-1. S-LDH is a valuable tumor marker in patients with TGCT, especially in those with seminoma. Routine determination of S-LDH isoenzymes in addition to S-LDH in patients with TGCT is not recommended. In patients with a history of TGCT and an unexplained elevation of S-LDH levels, a raised S-LDH-1 level indicates the presence of TGCT lesions.

Introduction

Serum activity of lactate dehydrogenase (L-Lactate: NAD⁺-oxidoreductase, EC 1.1.1.27), S-LDH, correlated significantly with the tumor load and the prognosis in patients with metastatic testicular germ cell tumors (TGCT) (von Eyben 1978; von Eyben et al. 1982a). Furthermore the application characteristics of S-LDH determination support the use of the test in these patients: S-LDH is a cheap method of analysis and routinely performed with quickly obtained results. S-LDH determinations according to the Scandinavian recommendation (The committee on enzymes of the Scandinavian society for clinical chemistry and clinical physiology 1974) have a high intra- and interlaboratory precision (Aronsson et al. 1980). All these factors indicate that S-LDH is a clinically useful tumor marker in patients with TGCT.

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The levels of anodal S-LDH isoenzymes, LDH-1 and LDH-2, were reported to be raised in 5 of 5 patients with seminoma (Zondag 1964). In another study, S-LDH-1 was the only raised S-LDH isoenzyme in 25 of 29 patients with non-seminomatous TGCT who had raised S-LDH (Boyle and Samuels 1977). However, in a third study, the levels of cathodal S-LDH isoenzymes, LDH-4 and LDH-5, were predominantly raised in 37 patients with TGCT (Akhyverdova and Nanobashvili 1977).

In the present study S-LDH and S-LDH isoenzymes were determined in patients with a history of TGCT. We describe the predictive value of S-LDH and S-LDH-1 with regard to the presence or absence of TGCT lesions, and the relation between S-LDH and S-LDH-1. Furthermore we evaluate the prognostic impact of normal and raised S-LDH and S-LDH-1 in the patients with metastatic TGCT.

Materials and Methods

Patients. Serum samples from 252 patients with a history of TGCT obtained between October 1979 and March 1980 were studied with respect to S-LDH and S-LDH isoenzymes. At diagnosis the median age was 33 years (range 15 to 81). None of the patients had megaloblastic or hemolytic anemia. All were followed until September 1982 at the Norwegian Radium Hospital.

At the time of the study, 37 had measurable TGCT lesions according to the results from clinical examination, roentgenograms, or computed tomograms. The histologic type of the primary tumor in these patients was revised according to the classification of the World Health Organization (Mostofi and Sobin 1976) for the purpose of this study. Table 1 shows the histologic type of the primary tumor, the previous treatment, and the clinical stage at the time of our study.

Two hundred and fifteen patients did not have TGCT lesions after previous treatment (orchiectomy, lymphadenectomy, radiotherapy, chemotherapy or combinations of these treatments). In these patients, the histologic type of the primary tumor was routinely reviewed at the time of the initial referral to the Norwegian Radium Hospital. One patient died due to hepatic failure shortly after the S-LDH isoenzyme determination. Autopsy showed extensive liver metastases from a colon cancer but no TGCT lesions.

Tumor Volume. We calculated the volumes of the tumor lesions as $\pi/6 \times \text{length} \times \text{width}^2$, the formula for an ellipsoid

Table 1. Patient characteristics in 37 patients with a history of TGCT and with TGCT lesions

	Number of patients
Histologic type of the primary tumor*	
Seminoma	11
Embryonal carcinoma	5
Yolk sac tumor	1
Choriocarcinoma	1
Mixed tumors	19
Stage at the time of the study	
Stage 1	3
Stage 2	14
Stage 3	20
Previous treatment	
No treatment	4
Orchiectomy with or without retroperitoneal lymphadenectomy	12
Orchiectomy and radiotherapy	2
Orchiectomy and chemotherapy	9
Orchiectomy, radiotherapy, chemotherapy with or without retroperitoneal lymphadenectomy	10

* Tumors classified according to the classification of the World Health Organization (Mostofi and Sobin 1976)

of revolution. The sum of the tumor volumes in each patient was used as an estimate of the total tumor mass.

S-LDH and S-LDH Isoenzyme Determinations. Blood samples were obtained from a peripheral arm vein in all patients. The sera were stored at -20°C until analysed for S-LDH and S-LDH isoenzymes. The S-LDH and S-LDH isoenzymes were redetermined in 30 samples after further storage for 8 months.

The S-LDH activity was determined according to the Scandinavian recommendation (The committee on enzymes of the Scandinavian society for clinical chemistry and clinical physiology 1974). We used $8.0\ \mu\text{kat/l}$ ($480\ \text{U/l}$) as the upper reference limit of S-LDH activity.

After electrophoretic separation followed by formazan staining the S-LDH isoenzyme activities were visually estimated (Lundh 1967; Niklasson et al. 1981). The upper reference limit of S-LDH isoenzyme activity for this study was calculated as the mean plus twice the standard deviation of the isoenzyme activities in the samples from our 214 patients with a history of TGCT but without tumor lesions (excluding the patient with colon cancer and liver metastases). Thus, the upper reference limit of S-LDH-1 was $3.0\ \mu\text{kat/l}$ ($180\ \text{U/l}$), of S-LDH-2 $3.1\ \mu\text{kat/l}$ ($184\ \text{U/l}$), of S-LDH-3 $2.2\ \mu\text{kat/l}$ ($131\ \text{U/l}$), of S-LDH-4 $0.2\ \mu\text{kat/l}$ ($10\ \text{U/l}$), and of S-LDH-5 $0.1\ \mu\text{kat/l}$ ($8\ \text{U/l}$).

Determination of Serum Alpha Fetoprotein (S-AFP) and Serum Human Chorionic Gonadotropin (S-hCG). S-AFP was measured with a radioimmunoassay. The upper reference limit of S-AFP was $20\ \mu\text{g/l}$. At the time of the first S-LDH determination for this study, S-AFP was measured in 33 of the 37 patients with TGCT lesions. S-hCG was measured with a beta subunit radioimmunoassay. The upper reference

limit of S-hCG was $10\ \text{U/l}$. S-hCG was determined in 30 of the 37 patients with TGCT lesions. The upper reference limit of S-AFP and S-hCG were determined in a series of healthy men.

Statistics. When more than one blood sample was obtained from a patient during the study period, the determination in the first sample were used as the patients were compared. We used all available determinations as we compared S-LDH and S-LDH isoenzymes with tumor volume.

The predictive value of a positive test (for instance raised S-LDH), the predictive value of a negative test (for instance normal S-LDH) (Vecchi 1966), the effectiveness of the test (for instance S-LDH) (Werner et al. 1973), the t-test (Armitage 1971), the Spearman rank correlation coefficient (Siegel 1956), the hypergeometric distribution, the Mann-Whitney U test (Siegel 1956), and the logrank test (Peto et al. 1977) were carried out for statistical analysis of the results.

Results

S-LDH

Fifteen of 37 (41%) patients with TGCT lesions had raised S-LDH. These patients had raised S-LDH levels significantly more often than those without TGCT lesions (7 of 215 (3%), $p < 0.001$). The histologic type of the primary tumor, the stage, and the previous treatment did not significantly influence the proportion of the patients with TGCT lesions who had raised S-LDH (Table 2).

The predictive value of raised S-LDH among the patients with a history of TGCT with regard to the presence of TGCT lesions was 0.68. The predictive value of normal S-LDH with regard to the absence of TGCT lesions was 0.90. The effectiveness of the test was acceptable: 89% of the patients had either TGCT lesions and raised S-LDH or no TGCT lesions and normal S-LDH.

Three of 7 patients without TGCT lesions who had raised S-LDH had other diseases known to be associated with raised S-LDH levels: one had colon cancer and liver metastases, one had chronic alcoholism, and one non-tropical sprue of the adult. The remaining four had raised levels of S-LDH in the first determination but normal S-LDH in the following analyses. None of these seven patients had a relapse during the follow-up.

The activities of S-LDH were significantly higher in the patients with TGCT lesions than in those without ($p < 0.001$, Table 2). At the start of the study the maximal S-LDH was higher in the patients with stage 2 or 3 lesions than with stage 1 lesions (Table 2). The activities of S-LDH in patients with raised S-LDH within each stage, however, did not differ significantly ($p > 0.05$).

S-LDH correlated significantly with the total tumor volume in the 37 patients with TGCT lesions (Spearman rank correlation coefficient $r = 0.46$, $p < 0.0005$) (Fig. 1), especially pronounced in those with seminoma ($r = 0.60$, $p < 0.0005$).

Of the 15 patients with TGCT lesions and raised S-LDH, two died shortly after the first serum sample. In two others, S-LDH levels were determined only once within the study period. Ten had concordant changes in S-LDH and tumor volume (1 shown in Fig. 2); however, one had a normalization of a slightly raised S-LDH activity after

Table 2. S-LDH in patients with a history of TGCT according to the patient characteristics

	Total number of patients	Number of patients with raised S-LDH	S-LDH ($\mu\text{kat/l}$)
Tumor status			
No TGCT lesions	215	7	5.2 (1.5-58.2)
TGCT lesions	37	15	7.5 (2.8-81.7)
Stage in patients with TGCT lesions at the time of the study			
Stage 1	3	2	10.2 (7.2-11.3)
Stage 2	14	4	5.8 (2.8-68.7)
Stage 3	20	9	7.5 (4.5-81.7)
Histologic type of the primary tumor in patients with TGCT lesions			
Seminoma	11	5	7.8 (4.5-68.7)
Embryonal carcinoma	5	2	7.5 (4.7-81.7)
Yolk sac tumor or choriocarcinoma	2	2	8.9 (8.3-9.5)
Mixed tumors	19	6	6.2 (2.8-31.7)
Previous treatment of patients with TGCT lesions			
No treatment	4	2	8.7 (5.2-11.3)
Orchiectomy with or without retroperitoneal lymphadenectomy	12	6	8.5 (2.8-68.7)
Orchiectomy and chemotherapy or radiotherapy or both with or without retroperitoneal lymphadenectomy	21	7	7.5 (4.5-81.7)

The results of S-LDH are given as the median and the range

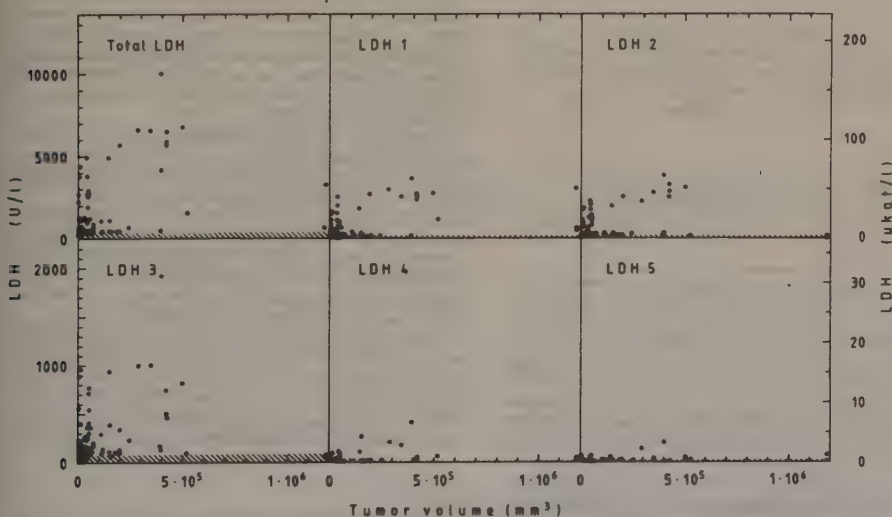


Fig. 1. S-LDH and S-LDH isoenzyme activities related to the total tumor volume of the patients. The marked area represents the range of normal activities

orchiectomy without an elevation of S-LDH later on when an abdominal tumor was found.

Of 34 patients with metastatic TGCT (stage 2 and 3) (Table 2), 13 with raised S-LDH lived a significantly shorter time than the 21 with normal S-LDH ($0.025 < p < 0.05$) (Fig. 3).

S-LDH Isoenzymes

Seventeen patients with TGCT lesions had at least one raised S-LDH isoenzyme; 15 had simultaneous S-LDH elevation, whereas two patients had normal S-LDH (Table 3). Four had only raised S-LDH-1 (one shown in Fig. 4f);

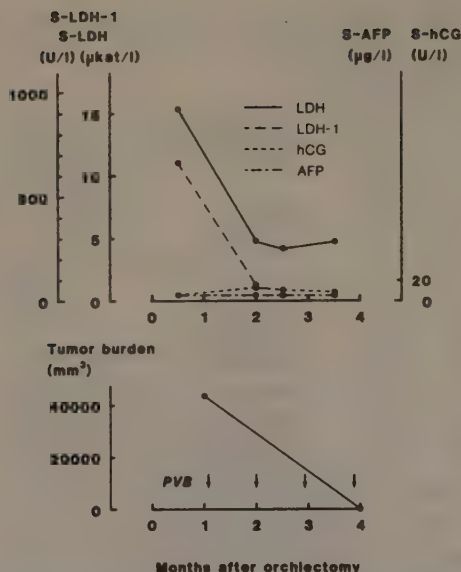


Fig. 2. Normalization of S-LDH and S-LDH-1 in 1 responding patient during chemotherapy with cisplatin, vinblastine, and bleomycin (PVB) after orchiectomy

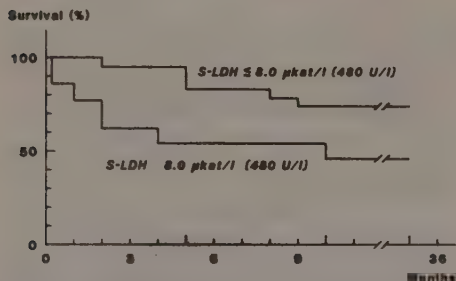


Fig. 3. Survival of 34 patients with TGCT metastases in relation to S-LDH (normal versus raised S-LDH, $0.025 < p < 0.05$)

Table 3. S-LDH and S-LDH isoenzymes in 252 patients with a history of TGCT

Tumor status	Total number of patients	Number of patients with normal S-LDH	Number of patients with raised S-LDH	Number of patients with raised S-LDH isoenzymes				
				S-LDH-1	S-LDH-2	S-LDH-3	S-LDH-4	S-LDH-5
No TGCT lesions	215	208	0	0	0	1	4	2
		0	7	6	7	4	2	4
TGCT lesions	37	22	0	0	1	1	0	0
		0	15	13	10	9	5	3

Upper reference limit of S-LDH is 8.0 µkat/l (480 U/l), S-LDH-1 3.0 µkat/l (180 U/l), S-LDH-2 3.1 µkat/l (184 U/l), S-LDH-3 2.2 µkat/l (131 U/l), S-LDH-4 0.2 µkat/l (10 U/l), and S-LDH-5 0.1 µkat/l (8 U/l)

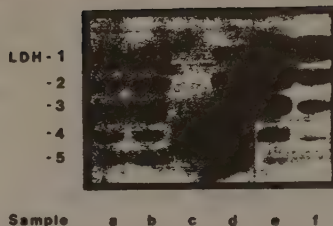


Fig. 4a-f. S-LDH isoenzymes in a normal control (a), and in patients with a history of TGCT, either without TGCT lesions (b-d) or with TGCT lesions (e and f). a and b show normal S-LDH isoenzyme activities; c shows an abnormal isoenzyme between LDH-4 and LDH-5; d-f show raised activities of the usual 5 LDH isoenzymes

one (with normal S-LDH) had raised S-LDH-2, two only raised S-LDH-3 (one with normal S-LDH), and 10 had five combinations of raised S-LDH isoenzymes with a predominance of S-LDH-1 and S-LDH-2 (one shown as Fig. 4e). Patients with TGCT lesions (13 of 37 (35%)) had raised S-LDH-1 significantly more often than those without (six of 215 (3%)) ($p < 0.01$).

In addition, six of the seven patients without TGCT lesions but with raised S-LDH had predominantly raised anodal S-LDH isoenzymes (Table 3). However, none of these patients had only raised S-LDH-1. The remaining patient, who had colon cancer and liver metastases, had normal S-LDH-1 but raised cathodal S-LDH isoenzymes, especially S-LDH-5 (Fig. 4d).

Regarding S-LDH-1, the predictive value of a raised activity with regard to the presence of TGCT lesions, the predictive value of a normal activity, and the effectiveness of the test were largely the same as those of S-LDH.

Of the patients with TGCT lesions and raised S-LDH, two of two with stage 1, one of four with stage 2, and one of seven with stage 3 had only raised S-LDH-1 ($p < 0.001$) (Table 4). Neither the histologic type of the primary tumor or the previous treatment had a major impact on the patterns of raised S-LDH isoenzymes (Table 4).

The activities of S-LDH-1 and S-LDH-2 were significantly higher in the patients with TGCT lesions than in those without ($p < 0.05$) (Table 5). S-LDH-3, S-LDH-4, and S-LDH-5 differed only slightly between the two groups (Table 5). S-LDH-1 correlated significantly with the total tumor volume in the 37 patients with TGCT lesions (Spear-

Table 4. S-LDH isoenzyme patterns according to the patient characteristics

	Total number of patients	Number of patients with normal S-LDH	Number of patients with raised S-LDH	Number of patients with raised S-LDH isoenzymes				
				S-LDH-1	S-LDH-2	S-LDH-3	S-LDH-4	S-LDH-5
Stage of the patients								
Stage 1	3	1	0	0	0	0	0	0
		0	2	2	0	0	0	0
Stage 2	14	10	0	0	1	0	0	0
		0	4	4	3	1	0	0
Stage 3	20	11	0	0	0	1	0	0
		0	9	7	7	8	5	3
Previous treatment								
No treatment or only surgical treatment	16	8	0	0	0	0	0	0
		0	8	8	4	2	1	1
Radiotherapy or chemotherapy or both	21	14	0	0	1	1	0	0
		0	7	5	6	7	4	2
Histologic type of the primary tumor								
Seminoma	11	6	0	0	1	0	0	0
		0	5	5	4	3	2	0
Embryonal carcinoma	5	3	0	0	0	0	0	0
		0	2	2	2	2	2	2
Other types	21	13	0	0	0	1	0	0
		0	8	6	4	4	1	1

Table 5. S-LDH isoenzymes in 252 patients with a history of TGCT

Tumor status	Total number of patients	Number of patients with normal S-LDH	Number of patients with raised S-LDH	S-LDH-1 ($\mu\text{kat/l}$)	S-LDH-2 ($\mu\text{kat/l}$)	S-LDH-3 ($\mu\text{kat/l}$)	S-LDH-4 ($\mu\text{kat/l}$)	S-LDH-5 ($\mu\text{kat/l}$)
No TGCT lesions	215	208	7	1.6 (0-26.2)	2.0 (0.3-29.1)	1.5 (0-10.6)	0.1 (0-5.6)	0.1 (0-33.4)
TGCT lesions	37	22	15	2.4 (0.8-61.8)	2.5 (0.1-32.7)	1.8 (0.8-15.5)	0.1 (0-1.6)	0.1 (0-0.9)

Results given as the median and the range

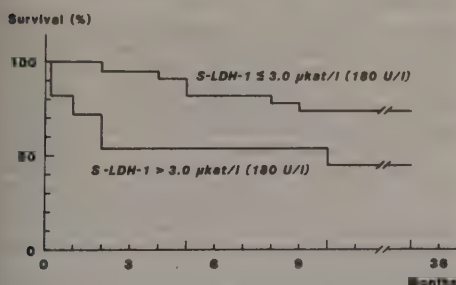


Fig. 5. Survival of 34 patients with TGCT lesions in relation to S-LDH-1 (normal versus raised S-LDH-1, $0.025 < p < 0.05$)

man rank correlation coefficient $r=0.46$, $p<0.0005$) (Fig. 2), especially in those with seminoma ($r=0.59$, $p<0.005$).

Of 11 patients evaluated for changes in S-LDH and tumor volume, 10 had raised S-LDH-1. S-LDH-1 and S-

LDH changed concordantly with the changes in tumor volume in nine, whereas one had normalization of raised S-LDH-1 and S-LDH levels after orchiectomy without subsequent elevation when an abdominal tumor was found later.

The survival of the patients with TGCT metastases with normal versus raised S-LDH-1 (Fig. 5) was much the same as the survival of those with normal versus raised S-LDH ($p<0.05$).

The usual five S-LDH isoenzymes were found in the samples from 251 of the patients (Fig. 4b, 4d, 4e, and 4f). One without TGCT lesions had normal S-LDH but an abnormal S-LDH isoenzyme localized between LDH-4 and LDH-5 (Fig. 4c).

The activities of S-LDH and S-LDH isoenzymes in the second determination of 30 samples after further frozen storage for eight months did not differ significantly from those of the first determination ($p>0.05$).

S-LDH, S-LDH-1, S-AFP, and S-hCG

In about 75% of the patients with TGCT lesions a concordance between raised and normal S-LDH, S-LDH-1, S-

Table 6. S-LDH, S-LDH-1, S-AFP, and S-hCG according to the histologic type of the primary tumor in 37 patients with TGCT lesions

	Total number of patients	Number of patients with normal S-LDH	Number of patients with raised S-LDH	Number of patients with raised S-LDH-1	S-AFP			S-hCG		
					<i>n</i>	<i>m</i>	<i>r</i>	<i>n</i>	<i>m</i>	<i>r</i>
Seminoma	11	6 0	0 5	0 5	5 4	1 0	0 1	5 3	1 0	0 2
Embryonal carcinoma	5	3 0	0 2	0 2	2 0	0 0	1 2	1 0	1 0	1 2
Other histologic patterns in the primary tumor	21	13 0	0 8	0 6	6 1	1 2	6 5	8 1	1 4	4 3

n denotes normal serum concentration, *r* raised serum concentration, and *m* no determination of the serum concentration at the time of the first S-LDH determination for the study

Table 7. Number of survivors in 34 patients with TGCT metastases according to the initial level of S-LDH, S-AFP, or S-hCG

	S-AFP			S-hCG			Survivors/the total number of patients
	<i>n</i>	<i>r</i>	<i>m</i>	<i>n</i>	<i>r</i>	<i>m</i>	
S-LDH <i>n</i>	13	6	2	13	5	3	16/21
<i>r</i>	5	6	2	4	6	3	6/13
Survivors/the total number of patients	16/18	4/12	2/4	13/17	5/11	4/6	22/34

n denotes normal serum level, *r* raised serum level, and *m* that the serum level was not determined. Three patients with stage I survived; three of these patients had raised S-AFP, one, raised S-hCG, one, normal S-hCG, and S-hCG was not determined in one patient

Table 8. Number of survivors in 34 patients with TGCT metastases according to the combined results of S-LDH, S-AFP, and S-hCG

	S-LDH		Survivors/the total number of patients
	<i>n</i>	<i>r</i>	
S-AFP and S-hCG <i>n</i>	11	0	10/11
	0	3	3/3
S-AFP or S-hCG <i>r</i>	5	0	2/5
	0	2	1/2
S-AFP and S-hCG <i>r</i>	2	0	2/2
	0	5	0/5
S-AFP or S-hCG or both <i>m</i>	3	0	2/3
	0	3	2/3

n denotes normal serum level, *r* raised serum level, and *m* that the serum level was not determined

AFP, and S-hCG was found, whereas a discordance between S-LDH and S-AFP and S-hCG was found in the remaining 25% (Tables 6-8).

Only one of the 10 patients with lesions from seminoma had raised S-AFP level (immunoperoxidase staining for AFP of the primary tumor was not carried out) and two had raised S-hCG (S-AFP and S-hCG were not determined in the 11th patient with seminoma lesions). In contrast, 5 of these patients had raised S-LDH.

Considering S-LDH, S-AFP, and S-hCG as single tests, the patients with TGCT metastases and normal S-LDH, normal S-AFP, and normal S-hCG survived more often

than those with raised serum levels. The difference in number of survivors, however, was only significant for S-AFP ($p=0.01$). The patients with normal S-LDH, S-AFP, as well as normal S-hCG had an excellent prognosis (10 survivors out of 11), whereas none of 5 with raised S-LDH, S-AFP, plus S-hCG survived (Table 8).

Discussion

S-LDH was raised in 15 of 37 patients with TGCT lesions at the first determination during the study period. The same proportion was found in another series (von Eyben et al. 1982a). A larger proportion was reported in a previous series in which the highest S-LDH activity of repeated determinations in each patient was evaluated (von Eyben 1978).

In the present study as in the two previous series (von Eyben 1978; von Eyben et al. 1982a), tumor volume and S-LDH activity correlated significantly in the patients with TGCT lesions. The correlation was especially pronounced in those with seminoma. The number of patients with other histologic types of TGCT was too small for separate analysis.

In this study and in the two previous series (von Eyben 1978; von Eyben et al. 1982a) the patients with TGCT metastases and raised S-LDH survived significantly shorter than those with normal S-LDH. S-LDH-1 had the same predictive value.

S-LDH level may be raised in patients with diseases other than TGCT (Cohen et al. 1966), but such disorders were not found in our patients with TGCT lesions. Seven of 215 patients with a history of TGCT but without TGCT

lesions had raised S-LDH. This proportion (3.3%) is close to the statistically expected 2.5% and significantly lower than that in the patients with TGCT lesions. In these patients, the elevations of S-LDH were therefore related to the presence of tumor lesions and were not due only to the history of TGCT.

S-LDH activities did not differ between the patients with TGCT lesions in stage 1, 2, and 3. Thus the localization of the TGCT lesions had no major impact on the release of LDH from the lesions into the blood. The stages are defined according to the localization of the tumor lesions and not to the total tumor volume. Therefore the results according to the stage are consistent with the concept of a correlation between S-LDH and tumor volume.

The S-LDH activity did not differ between previously untreated patients with TGCT and those previously treated. Thus no effect of treatment on S-LDH was found apart from the impact on tumor volume.

Seventeen out of 37 patients with TGCT lesions had raised S-LDH isoenzymes. Four of the 15 patients with raised S-LDH had only raised S-LDH-1, an unusual S-LDH isoenzyme pattern. The remaining 11 had other raised activities of S-LDH isoenzymes, though most often together with raised S-LDH-1. Our findings contrast with a report of only raised S-LDH-1 and S-LDH-2 as the typical isoenzyme pattern in patients with TGCT lesions (Zondag 1964). None of our patients with TGCT lesions had predominantly raised S-LDH-4 and S-LDH-5 levels, the pattern reported in another report (Akhverdova and Nanobashvili 1977), or predominantly raised S-LDH-2, S-LDH-3, and S-LDH-4 levels, a pattern most often found in patients with other malignancies (Zondag 1964). Based on our study, most patients with TGCT lesions and raised S-LDH have combined patterns of raised anodal S-LDH isoenzymes or, though less frequently, an isolated elevation of S-LDH-1.

In six of the seven patients without TGCT lesions but with raised S-LDH, the anodal S-LDH isoenzymes were predominantly raised. None, however, had an isolated elevation of S-LDH-1. In the remaining patient the raised S-LDH might have been interpreted as a sign of TGCT lesions, but this interpretation was contradicted by the predominance of the cathodal S-LDH isoenzymes and a normal S-LDH-1 activity. Thus his S-LDH isoenzyme pattern was significantly different from those in our patients with TGCT lesions.

Raised S-LDH predominantly due to raised S-LDH-1 is also found in diseases other than TGCT, for example myocardial infarction, pernicious anemia, and renal infarction (Cohen et al. 1966). These diseases can be differentiated from TGCT by history, clinical examination, and other laboratory analyses, as well as the course of the disease including the changes in S-LDH and S-LDH-1.

The high activities of S-LDH and S-LDH-1 are probably due to the release of LDH isoenzyme(s) from the tumor tissue into the blood. This hypothesis is supported by the shown correlations between S-LDH or S-LDH-1 and tumor volume. Furthermore one patient had a total S-LDH activity of 53.3 μ kat/l (3,200 U/l) and an S-LDH-1 activity of 49.8 μ kat/l (2,900 U/l) in testicular vein blood at orchiectomy for a TGCT, whereas S-LDH and S-LDH-1 in blood from an arm vein were only 20% of these activities. Similar findings were reported in another study (von Eyben et al. 1982b). As LDH-1 is not the predominant LDH isoenzyme in normal testicular tissue, these observations suggest that

LDH (especially LDH-1) is released from TGCT lesions into the blood. This interpretation is supported by the finding of a high activity of LDH-1 in serum and tumor tissue from one patient with seminoma (Zondag 1964) and from one patient with a testicular yolk sac tumor (Takeuchi et al. 1979). Furthermore another TGCT transplanted to athymic mice had also a high tissue activity of LDH-1, and S-LDH-1 correlated markedly with tumor volume in the mice (von Eyben et al. 1982c).

S-AFP and S-hCG are widely used as tumor markers in the treatment and follow-up of patients with TGCT. As described elsewhere (Bosl et al. 1981) the present study further indicates that the S-LDH activity and the S-AFP and S-hCG concentrations are discordant in some patients with TGCT lesions. S-LDH and S-LDH-1 were raised in about half of the patients with seminoma lesions, who most often have normal S-AFP and S-hCG. Therefore S-LDH is an especially valuable tumor marker in these patients.

In conclusion our study indicates that S-LDH is valuable as tumor marker in addition to S-AFP and S-hCG in patients with TGCT. S-LDH is of particular clinical value in those with seminoma. In patients with TGCT lesions, raised total S-LDH is most often due to raised anodal S-LDH isoenzymes, especially S-LDH-1. In those with a history of TGCT and an unexplained elevation of S-LDH the activities of S-LDH isoenzymes could be determined: normal S-LDH-1 may be a indication against the presence of TGCT lesions; an isolated elevation of S-LDH-1 level may indicate the presence of TGCT lesions. As the changes of S-LDH and S-LDH-1 were concordant in the patients with TGCT and raised S-LDH-1, S-LDH isoenzyme determinations are not considered obligatory in addition to S-LDH in the routine monitoring of patients with TGCT lesions.

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Multivariate Analysis of Risk Factors in Patients with Metastatic Testicular Germ Cell Tumors Treated with Vinblastine and Bleomycin

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Key Words. Testicular germ cell neoplasms · Alpha-fetoprotein · Human chorionic gonadotropin · Lactate dehydrogenase · Immune peroxidase technique · Combination chemotherapy · Multivariate analysis · Prognosis

Abstract. 39 patients with testicular germ cell tumor metastases were treated with vinblastine intravenous bolus injection day 1 and day 36 and bleomycin intramuscularly twice weekly for 10 weeks. 8 patients had complete remission. 4 of these are alive with no evidence of disease for 91+ to 109+ months. Studied with Wilk's method for stepwise discriminant analysis tumor volume and performance status significantly predicted the patients who had complete remission. Studied with Cox's proportional hazard model for multivariate analysis hCG in the primary tumor, serum lactate dehydrogenase, performance status, and complete remission had a significant impact on survival. The information from the risk factors may be useful in a differentiated treatment of patients with testicular germ cell tumor metastases.

The initial reports in the 70s indicated that combination chemotherapy with vinblastine and bleomycin was better than the previous regimens in the treatment of patients with metastatic testicular germ cell tumors [1, 2]. So far no follow-up longer than 5 years after treatment with vinblastine and bleomycin has been reported [1, 2]. Later, the addition of cisplatinum to the combination increased the response rate and survival in these patients [3-

5]. Nevertheless, about 30–50% of the patients are not cured. Factors pointing out the patients who have a high risk of dying in spite of the effective treatment of today should be known when selecting patients for an even more intensive treatment. In the present study the impact of potential risk factors on the response and survival were evaluated in patients with metastatic testicular germ cell tumors treated with vinblastine and bleomycin and followed for 6+ to 9+ years.

Material and Methods

Patients

39 consecutive patients with metastatic testicular germ cell tumors admitted to the Department of Surgery, Finseninstitutet, Copenhagen, between January 1972 and September 1975 were studied. In childhood 4 patients had been treated for maldescended testes. All patients had undergone orchiectomy. The primary tumor was histologically verified in all patients. At diagnosis the median age was 31 years (range 17–70). After the orchiectomy 25 patients had received high voltage irradiation, and 4 chemotherapy. 2 had been treated with chlorambucil, methotrexate, vincristine, and actinomycin D, 1 with vincristine, cyclophosphamide, and methotrexate, and 1 with a combination of vinblastine, bleomycin, and high voltage irradiation for 4 weeks.

Drug Administration

The patients received median 10 mg/m² body surface vinblastine bolus injection intravenously day 1 and 36 (range 2–16 mg/m²) and median 9 mg/m² body surface bleomycin i.m. twice weekly (range 7–19 mg/m²). The treatment was continued for 10 weeks unless the patients had progressive disease or refused further treatment.

Histological Evaluation

The orchiectomy specimens had been fixed in 10% buffered formalin for 24–72 h and embedded in paraffin. Hematoxylin and eosin stained sections of all available tumor blocks from 38 patients were reviewed and the tumors were reclassified according to the WHO classification [6]. The primary tumor of 1 patient with embryonal carcinoma was not available for the review and immunohistochemical staining.

Immunohistochemical Staining

Two representative tumor blocks of the primary tumor in 38 patients were used in this study. Serial sections cut at 5 μ m were stained for alpha-fetoprotein (AFP) and human chorionic gonadotropin (hCG) using an indirect immunoperoxidase technique [7, 8]. Specific rabbit antisera against human AFP (Dako, Copenhagen, lot. 029 A, protein concentration 9.65 g/l, in dilution 1/40) and against hCG (Dako, protein concentration 60.0 g/l, in dilution 1/200) were used in addition to peroxidase-labelled swine-antirabbit immunoglobulin (Dako, lot. 119, in dilution 1/20). All antisera were diluted in a phosphate buffered saline (PBS) with a pH of 7.2 and containing 10% normal swine serum. For control staining the specific antisera against AFP or hCG were replaced by immunoglobulin from unimmunized rabbits (Dako, lot. 903, protein concentration 20 g/l, in dilution 1/40), by

PBS, and by solutions in which the antiserum against AFP was absorbed with human AFP-standard (Behringwerke, Hoechst, Denmark) and in which the antiserum against hCG was absorbed with hCG (Leo, Denmark). The specimens were counterstained with hematoxylin and mounted in Aquamount®. Only pronounced red-brown granular intracytoplasmic staining was registered as positive. All control stainings were negative.

We did not quantitate the number of positively stained cells or the staining reaction of the individual tumor cells.

Clinical and Biochemical Investigations

At the start of the treatment history and physical examination was obtained in all patients. Performance status was evaluated according to the Zubrod scale (PS 0 = normal activity, PS 1 = normal activity with effort, PS 2 = some waking hours in bed, PS 3 = more than 50% of waking hours in bed, and PS 4 = totally bedridden) [9]. Determination of hemoglobin, white blood cells, reticulocytes, platelets, leukocyte differential, serum creatinine, alkaline phosphatase, aspartate aminotransferase, bilirubin, protein, albumin, calcium, and uric acid were obtained in all patients. Chest roentgenogram was obtained in all patients, bipedal lymphangiogram in 23, and liver scan in 14 patients. Determination of hemoglobin, white blood cells, and platelets and chest roentgenogram were obtained biweekly. Follow-up radiograms of the retroperitoneal lymph nodes were performed at 4-week intervals.

Tumor Markers

Serum lactate dehydrogenase (S-LDH) was determined in all patients every 2nd week during the treatment by the method recommended by the Scandinavian Enzyme Committee [10] with minor modifications. A S-LDH activity above 450 U/l (7.5 μ kat/l) was considered abnormal in the age span of the patients. Urinary excretion of hCG was measured repeatedly in 31 patients by a radioimmunological method [11]. A urinary hCG excretion above 300 U/24 h was considered abnormal.

Staging of Metastatic Disease

A semiquantitative staging of testicular germ cell tumor metastases [2] defined minimal pulmonary disease as no more than five metastases in each lung with a diameter of any lesion not larger than 2.0 cm. Advanced pulmonary disease was defined as a mediastinal or hilar mass, malignant pleural effusion, a pulmonary metastasis with a diameter larger than 2.0 cm, or more than five metastases in each lung.

Minimal abdominal disease was defined as retroperitoneal lymph node metastases detected on lymphograms without a palpable abdominal mass or displacement of the ureter or vena cava inferior. Advanced abdominal disease was defined as a palpable abdominal mass, ureter displacement or obstructive nephropathy by enlarged lymph nodes, displacement of vena cava inferior, or an enlarged liver with metastases [2].

Disease in other organs was defined as metastases apart from the pulmonary and abdominal lesions.

Tumor Volume Estimations

The tumor volume of each measurable tumor lesion was estimated as $\frac{\pi}{6} \times \text{length} \times (\text{width})^2$, the formula for an ellipsoid of revolution. We used the sum of the volumes in each patient as an estimate of the total tumor volume.

Response Criteria

Complete remission (CR) was defined as the disappearance of any tumor lesion and cancer related symptom for at least 1 month. Partial remission (PR) was defined as a 50% decrease or more of the product of the greatest length and width (tumor area) of measurable tumor lesions and no sign of progression for at least 1 month. No change (NC) was defined as changes less than 50% in tumor areas for at least 1 month. Progression (PD) was defined as a 50% enlargement or more in any tumor area or the appearance of a new tumor lesion. Treatment failure (TF) included the patients who refused the treatment due to subjective side effects or died within the course of treatment.

Statistical Methods

The potential risk factors (table I) were studied for impact on CR by use of the Wilk's test [12]. Many risk factors (for instance seminoma in the primary tumor) could take on only 2 values (0 = absence, 1 = presence) in the analyses. Some factors (for instance serum LDH) could take on a continuum of possible values. The survival of the patients was calculated by the life table method. The survival of groups of patients was compared by use of the Lee-Desu test [13]. Cox's proportional hazard model was used for multivariate analyses of the potential risk factors [14]. Thus, the significance of the factors in prediction of the survival was evaluated simultaneously. Two-tailed tests were generally used.

Results

Pretreatment Characteristics

The patients with limited pulmonary disease had a median tumor volume of the pulmonary lesions of 4 cm³ (range 0.3–15). The patients with advanced pulmonary disease had a median tumor volume of 18 cm³ (range 2–436).

The monitoring of the abdominal lesions did not allow a similar comparison of the tumor volume in the two groups of the staging system.

Therapeutic Response

8 patients had CR, 5 PR, 12 NC, and 10 PD. 4 were TF. The patients with CR lived significantly longer than the other patients ($p = 0.005$, one-tailed test, fig. 1). The patients with PR did not live significantly different from those with NC, PD, or TF ($p > 0.85$, fig. 1). The median survival of the patients with CR was 109+ months (range 3–109+).

Factors Predicting CR to Chemotherapy

All long-term survivors were found among the patients with CR. We compared, therefore, the patients with CR with the other patients as we evaluated the impact of potential risk factors (table I) on the response to the treatment. Only tumor volume ($p = 0.015$) and performance status ($p = 0.006$) predicted significantly the patients who had CR.

Table I. Potential risk factors

Prior maldescended testis	Patient characteristics at start of vinblastine and bleomycin
Age at diagnosis	Metastatic involvement
Histology of the primary tumor	Minimal/advanced
Presence of AFP in the primary tumor	Pulmonary/abdominal/other organs
Presence of hCG in the primary tumor	Tumor volume
Disease-free interval after orchiectomy	Number of organs with disease
Prior radiotherapy	S-LDH
Prior chemotherapy	Urinary hCG
Dosage of vinblastine and bleomycin	Response to vinblastine and bleomycin
	Subjective toxicity to vinblastine and bleomycin
	Myelosuppression after vinblastine and bleomycin

Single Factors with Impact on Survival

4 patients with a mixed tumor containing choriocarcinoma (table II) did not live significantly different from 6 patients with only seminoma or 7 patients with only embryonal carcinoma ($0.75 < p < 0.90$). 11 patients with embryonal carcinoma and yolk sac tumor with teratoma or without, lived significantly longer than the other patients ($p = 0.018$), and longer than the patients with only embryonal carcinoma.

20 patients with AFP positive primary tumors lived significantly longer than 18 patients with AFP negative tumors ($p = 0.014$) (table II). 17 patients with hCG positive primary tumors did not live significantly different from 21 patients with hCG negative tumors (table II).

The patients with a normal performance status lived significantly longer than the patients with an impaired performance status ($p = 0.0001$, fig. 2). The patients with S-LDH of 450 U/l ($7.5 \mu \text{kat/l}$) or less lived significantly longer than the patients with higher S-LDH ($p = 0.026$, fig. 3). The other factors considered (table I) did not have a significant impact on the survival ($p > 0.05$).

Multivariate Analysis of Prognostic Factors

In multivariate analysis of the potential risk factors (table I) four factors had a significant impact on the survival: performance status ($p = 0.003$), S-LDH ($p = 0.009$), hCG in embryonal carcinoma or choriocarcinoma ($p < 0.05$), and CR ($p = 0.014$) (fig. 4). The arrows in figure 4 show the statistically significant relationships.

4 of 7 patients with tumors without hCG positive embryonal carcinoma or choriocarcinoma structures, with a tumor volume less than

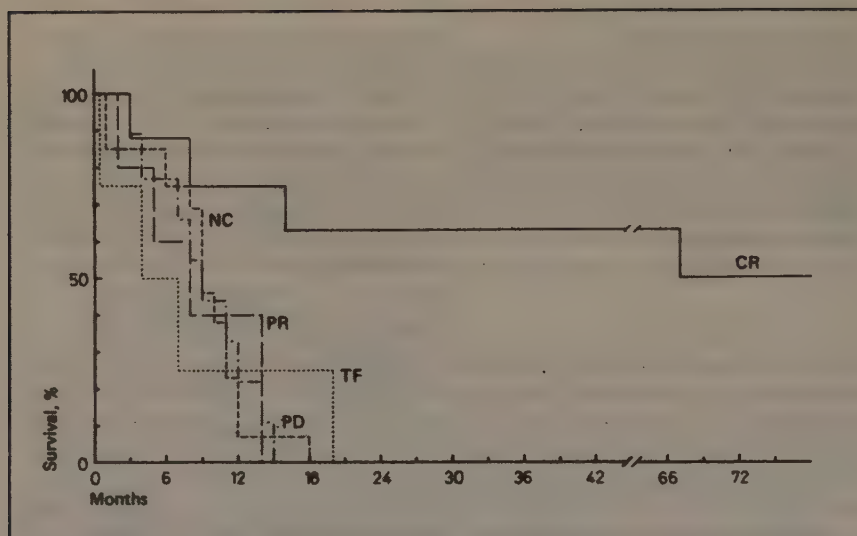


Fig. 1. Life table analysis of survival according to response to treatment with vinblastine and bleomycin. 8 patients had complete remission (CR), 5 partial remission (PR), 12 no change (NC), 10 progressive disease (PD), and 4 patients treatment failure (TF).

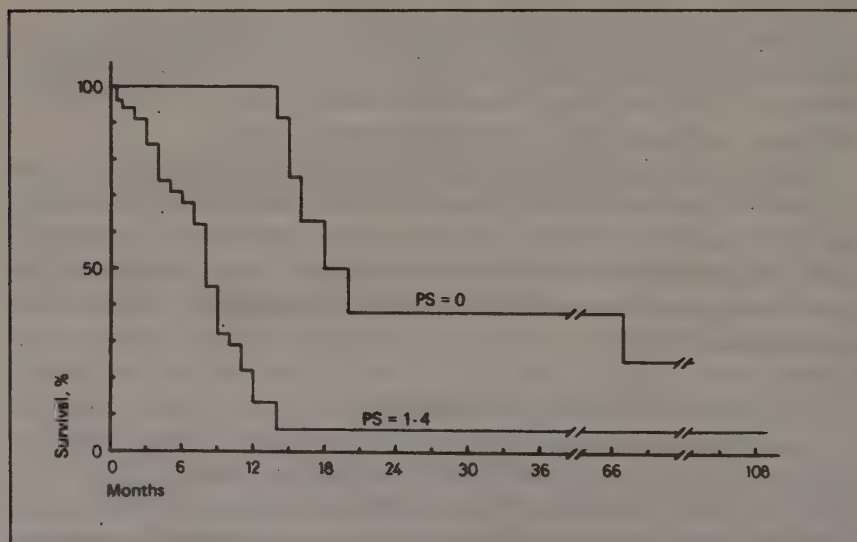


Fig. 2. Life table analysis of survival according to performance status. 8 patients had a normal performance status (PS = 0) and 31 impaired performance status (PS = 1-4).

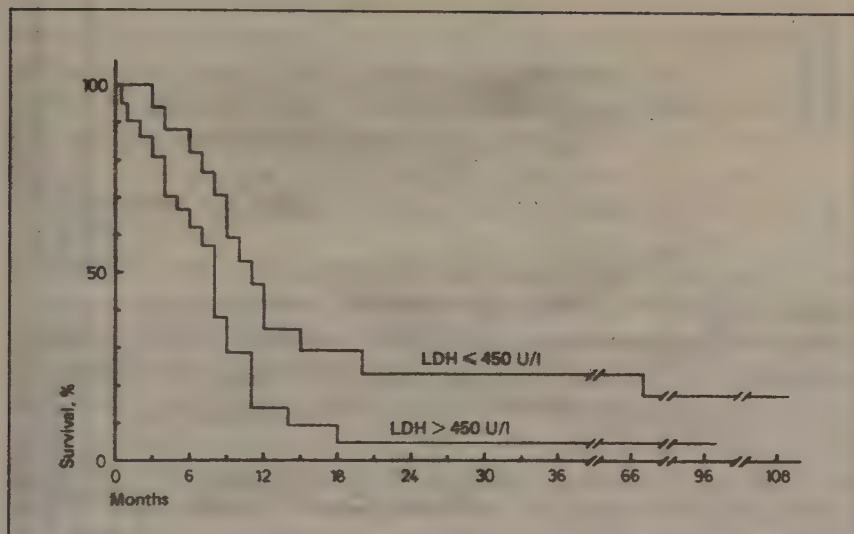


Fig. 3. Life table analysis of survival according to S-LDH at the start of treatment with vinblastine and bleomycin. 18 patients had a normal S-LDH (≤ 450 U/l) and 21 had a raised S-LDH.

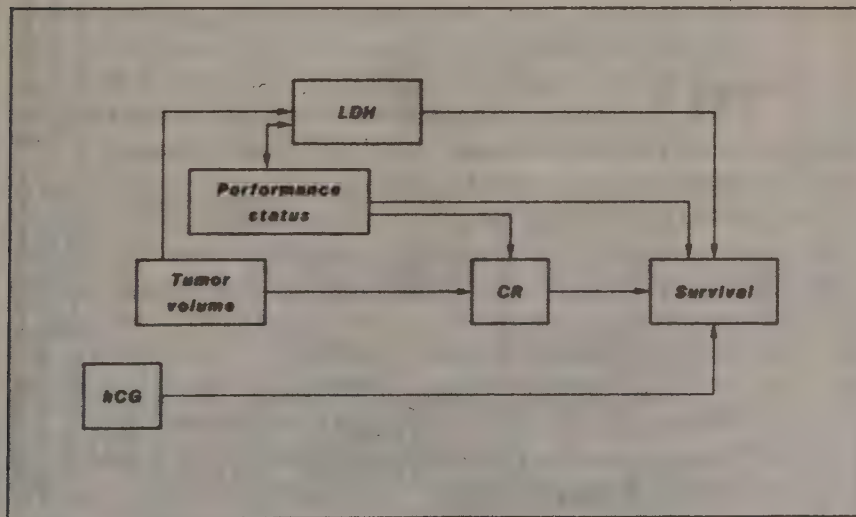


Fig. 4. Causal relationship between the factors found prognostically significant in multivariate analysis. As the analysis shows the association between the factors but not the relation of cause and effect, the directions of the arrows are based on clinical judgement.

Table II. Histochemical and immunohistochemical patterns in the primary tumors of the patients and their response to treatment with vinblastine and bleomycin

Histology	AFP staining		hCG staining				CR	Total number of patients ¹
	nega- tive	positive	nega- tive	positive				
				YST	non- YST	both		
Tumor of 1 histological type								
S	6	0	5	1	0	0	2	6
EC	3	0	6	0	0	0	1	6
T	1	0	1	0	0	0	0	1
Tumor of more than 1 histological type								
EC+T	2	0	1	2	0	0	0	3
CC+any types								
EC+CC	0	0	0	0	1	0	0	1
YST+CC	0	1	0	0	1	0	0	1
EC+CC+T	1	0	0	0	1	0	0	1
EC+YST+CC+T	1	0	0	0	1	0	0	1
Other combinations								
S+EC	1	0	1	1	0	0	1	2
S+YST	0	1	1	0	0	0	0	1
EC+YST	0	2	1	2	0	1	2	4
YST+T	1	0	1	0	0	0	0	1
S+EC+T	1	0	1	0	0	0	0	1
EC+YST+T	1	2	3	3	0	1	2	7
S+EC+YST+T	0	0	0	2	0	0	0	2

S = Seminoma; EC = embryonal carcinoma; T = teratoma; CC = choriocarcinoma; YST = yolk sac tumor; Non-YST = tumor patterns other than yolk sac tumor; both = yolk sac tumor and other histological patterns; STB = syncytiotrophoblastic giant cells.

¹ The primary tumor of a patient with embryonal carcinoma was not available for review of the histological pattern and immunohistological staining.

50 cm³, and either a normal performance status or a normal S-LDH or both lived more than 6 years. 2 patients with hCG positive embryonal carcinoma or choriocarcinoma structures in the primary tumor, with a tumor volume larger than 50 cm³, an impaired performance status, and a raised S-LDH died within 1 month.

Discussion

Results from multivariate analyses of risk factors may improve our understanding of the clinical behavior of testicular germ cell tumors compared with those of univariate analyses. In our study, the histological pattern of the primary tumors had a significant impact on survival in univariate analysis but not in multivariate: Only in univariate analysis the patients with embryonal carcinoma and yolk sac tumor with teratoma or without, and the patients with AFP positive tumors lived longer than the other patients. This implies a correlation between these histological components and the four factors that had a significant prognostic impact in multivariate analysis (performance status, S-LDH, hCG in the primary tumor, and CR). Furthermore, hCG positivity in embryonal carcinoma or choriocarcinoma had a prognostic significance in multivariate analysis but not in univariate.

The impact of performance status on survival has been previously reported both in patients with metastatic testicular germ cell tumors [4] and with other types of cancers (i.e. metastatic colorectal cancer) [15].

A prognostic value of S-LDH in patients with testicular germ cell tumors has been previously reported [16].

A marked correlation between tumor weight and volume calculated according to the formula for an ellipsoid of revolution was found in a study of a germ cell tumor xenografted to athymic mice [17]. So we calculated the tumor volumes by the formula in this study too. When measured quantitatively, the tumor volume of the patients had a prognostic significance, while a semiquantitative staging did not [2]. This may in part be due to the overlap of volume of the pulmonary metastases between the patients in the classes of minimal and advanced pulmonary disease.

The patients with primary tumors containing hCG positive syncytiotrophoblastic giant cells lived similar to the patients without hCG in the primary tumor, whereas the patients with hCG positive embryonal carcinoma or choriocarcinoma lived a shorter time than the other patients. In another study the patients with hCG positive tumors and hCG negative tumors had a similar prognosis [18].

As in other series, we found a close relationship between CR and long-term survival [2, 4, 5, 19]. But in our study a further three factors had a significant predictive value in multivariate analysis even when considering CR simultaneously. This implies a genuine role for these three factors in patients with testicular germ cell tumor metastases.

The significant prognostic factors in our study were also important when analysed as single factors in patients treated with a regimen of vinblastine, bleomycin, and cisplatin [4]. The semiquantitative classification of metastases [2] points out a group of patients that has a more than 50% risk of dying from their tumor in spite of the three-drug regimen [5]. Our risk factors may point out a group of patients with an even greater risk of dying, as they use the prognostic information from the risk factors more effectively than this classification. Such a group of patients may benefit from a more intensive treatment than the three-drug regimen. A more intensive treatment is not indicated in the patients who with the three-drug regimen have a more than 90% chance of long-term survival in CR [5].

In conclusion, the information from our risk factors may be useful in a differentiated treatment of the patients with testicular germ cell tumor metastases.

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SERUM LACTATE DEHYDROGENASE AND ITS ISOENZYMES IN MEN WITH MALDESCENDED TESTES

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ABSTRACT

Serum lactate dehydrogenase and lactate dehydrogenase isoenzymes were determined as a screen for testicular germ cell neoplasia in 130 men with maldescended testes. A testicular tumor was found on clinical examination in 1 patient, which was revealed to be embryonal carcinoma, teratoma, yolk sac tumor and carcinoma in situ on orchiectomy. Subclinical testicular germ cell neoplasia was found on testicular biopsy in 3 men (1 with microinvasive seminoma and 2 with carcinoma in situ). These 4 patients had normal serum lactate dehydrogenase and serum lactate dehydrogenase isoenzymes. Elevated serum lactate dehydrogenase was noted in 3 men without testicular germ cell neoplasia: 1 had predominantly increased serum lactate dehydrogenase isoenzymes 1 to 3 and 2 had slightly increased serum lactate dehydrogenase isoenzymes 3 and 4. Serum lactate dehydrogenase and lactate dehydrogenase isoenzymes were not sensitive to detect testicular germ cell tumors in a subclinical stage.

Maldescended testis is the most important of the known risk factors for testicular germ cell tumors. Approximately one-tenth of the patients with testicular germ cell tumors have maldescended testes.¹ The lifetime risk for testicular germ cell tumor in patients with maldescended testes has been estimated to be about 1 of 30.¹

A high incidence of testicular germ cell neoplasia was found in men with maldescended testes screened with testicular biopsies and clinical examination.² Serum α -fetoprotein (AFP) and human chorionic gonadotropin (HCG) are used widely as tumor markers in patients with testicular germ cell tumors. However, neither serum AFP nor HCG was increased in the 3 screened patients with subclinical testicular germ cell neoplasia.³

Serum lactate dehydrogenase (L-lactate:NAD⁺ oxidoreductase, EC 1.1.1.27, LDH) and the anodal serum isoenzymes LDH-1 and LDH-2 are used as tumor markers in patients with testicular germ cell tumors.⁴⁻⁶ The serum LDH isoenzyme patterns in patients with testicular germ cell tumors differ from those in patients with most other malignancies⁷ or other disorders.⁸ We herein evaluate whether serum LDH and LDH isoenzyme determinations might detect testicular germ cell neoplasia in men with maldescended testes. The results are related to 1) the presence or absence of testicular germ cell neoplasia in testicular biopsy specimens or by physical examination, 2) the determinations of serum AFP and HCG, and 3) the results of followup ranging from 1.5 to ≥ 4 years after the screening examination.

MATERIALS AND METHODS

We examined 130 men with maldescended testes between October 1976 and May 1979. Data from the Danish Cancer Registry until December 1980 were used as followup.

Determination of LDH and LDH isoenzymes. Blood samples were obtained from a peripheral arm vein. A concomitant blood sample was obtained at orchiectomy from the testicular vein on the side of the tumor in 1 patient. The sera were stored at -20°C until LDH and LDH isoenzymes were determined. A total of 75 blood samples had been thawed once for HCG determination. LDH was determined according to a recommended method.⁹ A serum LDH level >480 U./l. was considered abnormal in the age span of the patients.

After electrophoretic separation followed by formazan stain-

ing the serum LDH isoenzymes were estimated visually by 1 of us (G. S.) as described previously,^{9,10} without knowledge of whether the men had testicular neoplasia. The variation coefficient in visual estimation of serum isoenzymes is about 8 per cent.¹¹ The mean value ± 2 times the standard deviation of the serum LDH isoenzyme determinations was used as the upper limit of normal for serum LDH isoenzyme in 214 patients without evidence of disease after treatment for testicular germ cell tumor. This limit differed only slightly from the upper 2.5 percentile. Activities of serum LDH-1 >3.0 $\mu\text{kat./l.}$ (180 U./l.), LDH-2 >3.1 $\mu\text{kat./l.}$ (184 U./l.), LDH-3 >2.2 $\mu\text{kat./l.}$ (131 U./l.), LDH-4 >0.2 $\mu\text{kat./l.}$ (10 U./l.) and LDH-5 >0.1 $\mu\text{kat./l.}$ (8 U./l.) were considered abnormal.

Determination of serum AFP and HCG. Serum AFP was determined by a radioimmuno-electrophoretic method.¹² Serum HCG was determined by use of a radioimmunoassay technique.¹³

Testicular biopsy and determination of tumor volume. Testicular specimens from 76 of the 130 men were examined histologically as described previously.² A testicular specimen for biopsy was contraindicated in 1 patient with a macroscopic tumor.

RESULTS

Serum LDH. Of 126 men without testicular germ cell neoplasia at clinical examination 3 had increased and 123 had normal serum LDH levels. Testicular biopsies revealed subclinical testicular germ cell neoplasia in 3 men with normal serum LDH levels: 2 had carcinoma in situ² and 1 had microinvasive seminoma⁴ with a tumor volume of 3 cm.³. A macroscopic 34 cm.³ tumor was noted at physical examination in 1 patient and orchiectomy was done. Histological examination of the tumor revealed embryonal carcinoma, teratoma, yolk sac tumor and carcinoma in situ. At orchiectomy the patient had increased serum LDH levels in blood from the testicular vein and normal levels in blood from an arm vein.

Serum LDH isoenzymes. Of the 3 men with increased serum LDH levels 2 had increased serum LDH-3 and LDH-4, and normal serum LDH-1 and LDH-2 levels. Serum LDH-1 to LDH-3 isoenzyme values were increased moderately and the cathodal serum isoenzymes LDH-4 and LDH-5 were increased only slightly in the remaining man.

The serum LDH-1 to LDH-3 levels were increased in testic-

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ular vein blood in the patient with a macroscopic tumor, while they were normal in blood from an arm vein.

All men with normal serum LDH values had normal serum LDH isoenzyme levels.

Serum AFP and HCG. The patient with a macroscopic tumor had increased serum AFP and HCG values before orchiectomy.³ The 3 patients with and 126 men without subclinical testicular germ cell neoplasia had normal serum AFP and HCG levels.³

The diagnostic sensitivity (predictive value of a negative test)¹⁵ of serum LDH, anodal serum LDH isoenzymes, serum AFP and serum HCG was similar.

Testicular biopsy. None of the 3 men with increased serum LDH levels underwent testicular biopsy. These 3 men were followed for ≥ 4 years without detection of testicular germ cell tumor.

DISCUSSION

Several factors suggest a potential for testicular germ cell tumor screening in men with maldescended testes. Although testicular germ cell tumor is a relatively rare disease it is the most common cancer in men 20 to 35 years old.¹⁶ Testicular germ cell tumor in men with maldescended testes who did not undergo screening procedures implied treatments with many side effects.^{17,18} Approximately a fourth of the patients had widespread metastases at diagnosis and approximately 40 per cent died of tumor.^{17,18}

In all screened men serum LDH and LDH isoenzymes were determined with little inconvenience and no risk. The diagnostic sensitivity (the predictive value of a negative test)¹⁵ of serum LDH determination was high. None of the patients with subclinical testicular germ cell neoplasia had increased serum LDH levels.

Although patients with testicular germ cell tumors characteristically have increased anodal serum LDH isoenzymes,⁶ the serum LDH isoenzymes also were normal in the men with subclinical testicular germ cell neoplasia.

In contrast with our results, a previous report described increased serum LDH-1 and LDH-2 levels in a patient before a testicular germ cell tumor was proved histologically.⁵ A malignancy was suspected and nonmalignant diseases with increased serum LDH-1 and LDH-2 levels were excluded. After a short interval of cyclophosphamide treatment an abdominal mass was detected, serum LDH values increased and the patient died. Autopsy revealed a small teratoma in a testis and widespread metastases.

Testicular biopsy and clinical examination were important to screen for testicular germ cell neoplasia in men with maldescended testes. The disease was found in a localized or subclinical stage in 4 patients with testicular germ cell neoplasia. If serum LDH and LDH isoenzyme determinations had been used as the only screening procedures diagnosis would have been delayed in our patients with testicular germ cell neoplasia. Serum LDH and LDH isoenzymes, as well as serum AFP and HCG determinations should not be used as the only screening procedures.

The Danish Cancer Registry provided information used as followup of the men. Ulla Bengzon, Department of Clinical Chemistry, Central Hospital, Kalmar, provided technical assistance. Dr. S. Fischer, Frederiksberg Hospital, and Dr. N. E. Skakkebaek and Prof. J. Vissfeldt, Rigshospitalet, Copenhagen, provided information as to the histological findings.

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EDITORIAL COMMENT

Electrophoretically in man there are 5 heterogeneous isoenzymes called LDH. Cancer cells have increased glycolysis, leading to an increased synthesis of lactate. Therefore, these isoenzymes may be used as nonspecific tumor markers in several cancers, including that of testicular germ cell. However, these authors have investigated the value of these isoenzymes as a screening test for determination of malignancy in 130 men with cryptorchidism. Serum LDH was not sensitive enough to determine any neoplastic changes in these 130 patients, although examinations and biopsies revealed 4 patients with testicular cancer. Therefore, these authors have considered serum LDH an insensitive test for detection of testicular cancer in "subclinical stage." Our experience at the National Cancer Institute confirms the findings of these authors as far as small microscopic testicular cancer is concerned. However, serum LDH often is elevated in cases of bulky testicular cancer and is of value in monitoring and in prognosis of this tumor. We have reported previously from our clinical program for testicular cancer the value of serial serum LDH to be useful because of availability and simplicity of the assay.² Serum LDH is particularly helpful in monitoring the patients with bulky seminoma owing to the lack of frequency of other markers. Furthermore, in bulky nonseminomatous testicular cancer with normalization of serum AFP and HCG while the patient is on intensive chemotherapy, the serum LDH remains elevated with residual tumor and may be used to monitor further therapy. However, the authors have concluded correctly that, presently, clinical examination and biopsy are the most reliable methods of detecting small foci of malignancies in patients with undescended testes.

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REPLY BY AUTHORS

Previous experience confirms that serum LDH is of value in the monitoring and prognosis of patients with metastatic testicular germ cell tumors.^{2,4} Serum LDH-1 and LDH-2 were predominant in such patients with increased serum LDH levels, whereas increased serum LDH seldom is found in patients cured of testicular germ cell tumors.⁵ Similarly, LDH-1 was predominant in tissue from testicular germ cell tumors⁶ and in cell lines from nonseminomatous testicular germ cell tumors.⁷ Serum LDH related significantly to tumor volume, which may explain why determinations of serum LDH and the anodal LDH isoenzymes were useful in patients with testicular germ cell tumor metastases and insensitive tests in patients with subclinical testicular germ cell tumors.

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Lactate Dehydrogenase Isoenzyme 1 (LDH-1) in Athymic Mice with Xenografts of a Human Testicular Germ Cell Tumor

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Summary. Lactate dehydrogenase (LDH) and LDH isoenzyme activities were determined in tumor tissue, tumor cystic fluid, and serum from athymic mice with transplants of a human testicular germ cell tumor. High activity of LDH-1 was found in tumor tissue and tumor cystic fluid. By histochemical staining LDH was found in all tumor cells. Most tumor cells had a faint staining reaction while some cells dispersed throughout the tumor had a strong staining reaction. The serum LDH-1 in athymic mice with tumor transplants correlated markedly with the tumor volume. The results indicate that serum LDH-1 was derived from the testicular germ cell tumor transplants.

Introduction

Recent studies indicate that the activity of serum lactate dehydrogenase (LDH, EC 1.1.1.27, L-Lactate:NAD⁺ oxidoreductase) and LDH isoenzymes may be of use as tumor markers in patients with testicular germ cell tumor lesions (von Eyben 1978; Bosl et al. 1981; Lippert and Javadpour 1981). Serum LDH increases and decreases concordantly with changes in tumor volume in these patients (von Eyben 1978). A higher serum LDH activity was found in blood from the testicular vein on the side of the tumor compared with the LDH activity in blood from an arm vein in a patient at orchiectomy (von Eyben et al. 1982a). The anodal LDH isoenzymes, LDH-1 and LDH-2, were predominantly raised in serum and dominant in tumor tissue from a patient with seminoma (Zondag 1964). In contrast LDH-4 and LDH-5 were predominantly raised in serum and dominant in tumor tissue in another series of patients with testicular germ cell tumors (Akhverdova and Nanobashvili 1977). LDH-1 was predominant in serum and tumor tissue from a patient with a yolk sac tumor as well as in serum and tumor tissue from athymic mice with transplants of this tumor (Takeuchi et al. 1979).

Due to the contradictory findings in the previous reports we studied LDH and LDH isoenzyme activities in serum and tumor tissue from athymic mice with transplants of a human testicular germ cell tumor.

Materials and Methods

Tumors. A tumor specimen from a pulmonary metastasis of embryonal carcinoma, teratoma, and yolk sac tumor in a man with an advanced testicular germ cell tumor was transplanted to athymic mice in August, 1979 (von Eyben et al. 1982b). After a few passages in the athymic mice, the tumor showed an embryonal carcinoma and teratoma by light microscopical examination and had no reactivity for alpha-fetoprotein or human chorionic gonadotropin by indirect immunoperoxidase staining. Tumor specimens and serum samples from athymic mice with tumors from the 13th-26th passage were obtained for this study between October 1980 and December 1981.

Heterotransplantation. BALB/c nu/nu athymic mice were bred in our laboratory from nu/+ heterozygous mice obtained from the Laboratory and Research Center, Bomholtsgaard, Ry, Denmark. Male and female athymic mice were 5-8 weeks old when implanted. The mice were kept in sterile cages, but not under specific-pathogen-free conditions. Tumor specimens measuring about 4 mm³ were implanted subcutaneously in the back area of the mice.

Measurements of Tumor Volume, Tumor Growth, and Tumor Weight. The length and width of the tumors were measured immediately before the mouse was killed. The tumor volume was estimated by $\frac{\pi}{6} \times \text{length} \times (\text{width})^2$, the formula for an ellipsoid of revolution. The growth of the tumors was analyzed using the Gompertz equation

$$N(t) = N(0) \left\{ \exp^{\alpha} (1 - \exp^{-\beta t}) \right\}$$

where $N(t)$ is the tumor volume at time t , $N(0)$ the tumor volume at time zero, α the initial growth rate, and β the retardation factor (von Eyben et al. 1982). The tumor weight was measured immediately after the tumor was extirpated.

Histological Examinations of the Tumors. Tumor specimens were fixed in Bouin's solution, embedded in paraffin, cut at 5 μ m, and stained with hematoxylin-eosin stain for light microscopical examination.

A small part of each of the tumor specimens was fixed in 4% glutaraldehyde with 0.2 M phosphate buffer, stained in 2% osmium tetroxide and embedded in Vestopal. UI-

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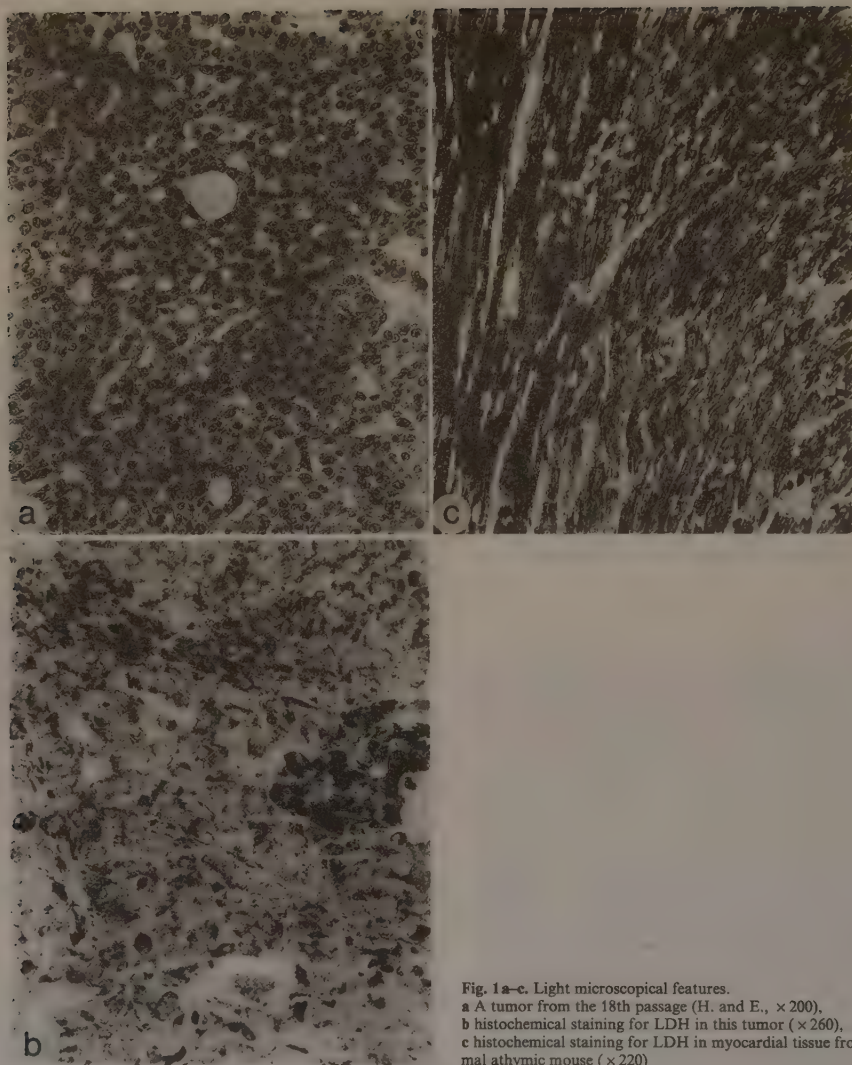


Fig. 1a-c. Light microscopical features.
 a A tumor from the 18th passage (H. and E., $\times 200$),
 b histochemical staining for LDH in this tumor ($\times 260$),
 c histochemical staining for LDH in myocardial tissue from a normal athymic mouse ($\times 220$)

trathin sections stained with uranyl acetate and lead acetate were examined under a Philips 300 electron microscope.

Determination of LDH and LDH Isoenzymes in Mouse Serum and Tumor. About 0.5 ml blood was aspirated from the ventricles of the heart immediately after the mouse was killed. The blood coagulated for 1 h and was centrifuged for 5 min at 3,000 rpm. The serum was frozen and kept

at -20°C . The LDH activity was determined according to the Scandinavian recommendation (The committee of enzymes of the Scandinavian society for clinical chemistry and clinical physiology, 1974). After electrophoretic separation followed by formazan staining (Lundh 1967) the LDH isoenzymes were visually estimated.

The tumors obtained for LDH isoenzyme determination were frozen at -20°C immediately after they were extir-

pated. Homogenates of the tumors were prepared in a phosphate buffer containing 154 mM NaCl and subjected to electrophoretic separation.

Histochemical Determination of LDH in Tissue. Tissue from tumors of the 18th passage and tissue from liver and skeletal muscle – with a predominance of LDH-5 – and from the heart – with a predominance of LDH-3 – of a normal athymic mouse without tumor transplants were stained histochemically for LDH (Pearse 1972). At pH 7.4 all positive staining is due to LDH activity.

Results

Histological Examinations

Light microscopical examination of the lung metastasis revealed mainly epithelial patterns with elongated and branched solid structures. No differentiated structure or trophoblastic element was found. The metastasis was an undifferentiated malignant teratoma according to the classification of the British Testicular Tumour Panel (Pugh and Cameron 1976).

Examinations of tumor tissue from the 13th–21st passage revealed undifferentiated, malignant tumors. Solid and reticular patterns were predominant in many tumors of these passages. Tubular and glandular structures were sparsely seen. The mitotic activity was high in all tumors (Fig. 1a).

Histochemical Staining for LDH

Histochemical staining revealed LDH activity in all tumor cells. While most tumor cells were stained faintly, some cells dispersed all through the tumor were stained strongly (Fig. 1b). No concentration of these cells close to necrotic areas in the tumors was found. On light microscopical examination the strongly stained and the weakly stained cells were similar. The LDH staining in the faintly stained tumor cells was weaker than that of the myocardial cells from a normal athymic mouse (Fig. 1c).

Ultrastructural Examinations

Ultrastructural examinations of a tumor from the 18th passage revealed tumor cells with large nuclei with chromatin adherent to the nuclear membrane (Fig. 2a). Desmosomes between tumor cells were found (Fig. 2b). The tumor cells had numerous free ribosomes, as well as endoplasmic reticulum and Golgi apparatus in the cytoplasm (Fig. 2c). No evidence of LDH producing virus was found.

Tumor LDH Isoenzyme Pattern

High activity of LDH-1 was found in six out of eight tumor homogenates from tumors of the 13th and 16th passage. In two tumors from the 13th passage LDH-1 was only moderately active. LDH-1 was the dominant LDH isoenzyme in cystic fluid from four out of four tumors of the 13th passage.

In addition, a high activity of mouse LDH-5 was found in all tumors of the 13th and 16th passage.

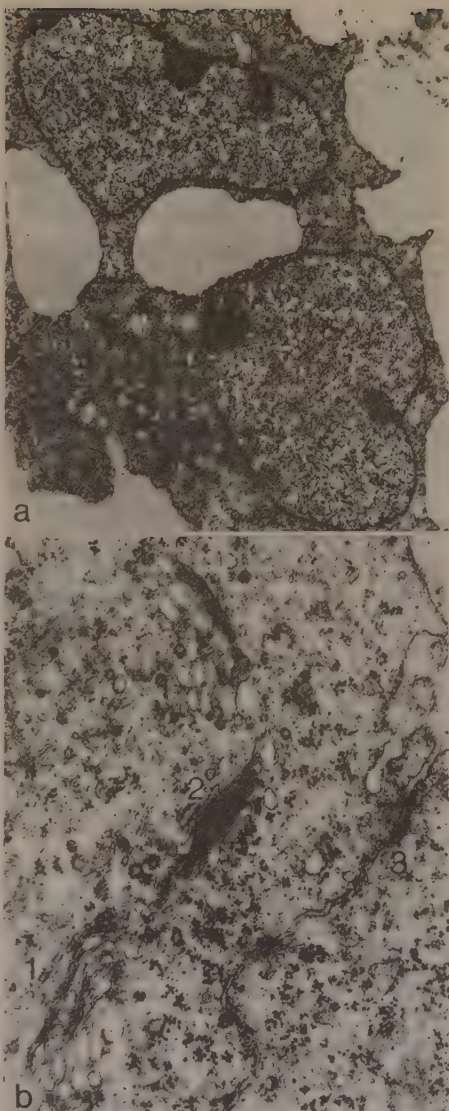


Fig. 2a, b. Ultrastructural features of a tumor from the 18th passage. a Two tumor cells ($\times 7,100$), b numerous organelles in the cytoplasm of the tumor cells (1 indicates golgi apparatus, 2 endoplasmic reticulum, and 3 desmosome) ($\times 32,500$)

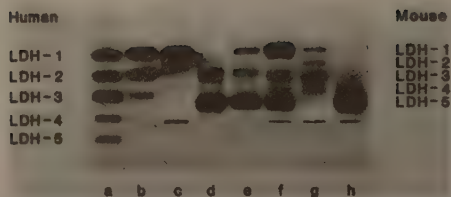


Fig. 3a-g. LDH isoenzyme patterns. a Serum from a normal man, b serum from a patient with a seminoma, c homogenate from a testicular germ cell tumor. d serum from a normal athymic mouse, e serum from an athymic mouse with a tumor xenograft (940 mg), f serum from an athymic mouse with a tumor xenograft (2,422 mg), g homogenate of heart tissue from a normal athymic mouse, h homogenate of liver tissue from a normal athymic mouse

Mass Density of the Tumors

The mass density of the tumors were grossly stationary in the studied passages (median 0.42 mg/mm³, range 0.27-0.59).

Growth of Tumor

Only local growth of the tumors was found at macroscopic examination of the killed mice. In two tumors of the 21st passage the initial growth rate was 0.2065 and 0.2507 and the retardation factor was 0.0412 and 0.0526.

LDH Isoenzymes in Tissues from a Normal Athymic Mouse

In heart from a normal athymic mouse LDH-3 activity was predominant. The LDH-1 and the LDH-2 activities were moderate (Fig. 3g).

In liver tissue from a normal athymic mouse, high activity of the mouse LDH-5 was found (Fig. 3h).

In muscle tissue the activity of mouse LDH-5 was high and the activity of LDH-1 and LDH-2 low.

Serum LDH and LDH Isoenzymes in Normal Athymic Mice

In three athymic mice without tumor transplants, the serum LDH activity was 800, 1,700, and 7,200 U/l. In the electrophoretic system mouse LDH-1 migrated similarly to human LDH-1. Mouse LDH-5 was localized between human LDH-3 and LDH-4. About 97% of the serum LDH migrated as mouse LDH-3 and LDH-5. Only about 3% of the serum LDH migrated as LDH-1 (24-210 U/l) (Fig. 3d).

Serum LDH and LDH-1 in Xenografted Athymic Mice

The serum LDH activity varied in the athymic mice with tumor transplants (median LDH 3,200 U/l, range 840-11,600 U/l). The variation of LDH was mainly due to variation of the mouse LDH-5 (median 2,600 U/l, range 780-10,700). Neither the activity of the total LDH nor the activity of the mouse LDH-5 correlated with the tumor weight (correlation coefficient $r = 0.01$ and -0.19).

In 9 of 15 athymic mice whose tumor weights were less than 0.9 g, serum LDH-1 was above 210 U/l. In seven of seven athymic mice whose tumor weights were 0.9 g or

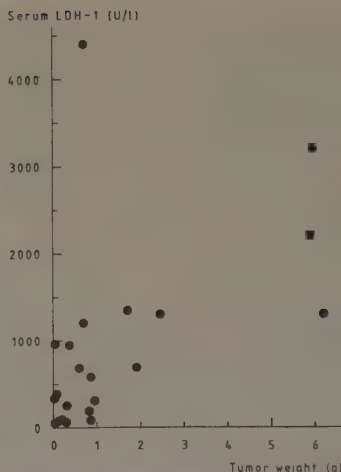


Fig. 4. Serum LDH-1 activities and tumor weights in athymic mice with xenografts of a testicular germ cell tumor

more, serum LDH-1 was above 210 U/l (Fig. 3e and 3f). Serum LDH-1 correlated markedly with tumor volume (correlation coefficient $r = 0.51$) and tumor weight ($r = 0.52$) (Fig. 4).

LDH Isoenzymes in Patients with Testicular Germ Cell Tumors

Serum LDH-1 is the LDH isoenzyme most often raised in patients with testicular germ cell tumors (Fig. 3b) compared with the LDH isoenzyme pattern in normal men (Fig. 3a). Correspondingly the LDH-1 activity was predominant in homogenates of a human testicular germ cell tumor (Fig. 3c).

Discussion

A marked correlation between serum LDH-1 and tumor weight was found in this study of a testicular germ cell tumor transplanted to athymic mice. Tumor tissue had a high activity of LDH-1, and LDH-1 was the dominant LDH isoenzyme in tumor cystic fluid. These results indicate that the tumors released LDH-1 into the blood of the athymic mice. Corresponding to the correlation between serum LDH-1 and tumor volume, histochemical staining revealed a high LDH activity in some cells dispersed all through the tumors.

The serum LDH activity in athymic mice with or without tumor transplants varied mainly due to the variation in the activity of mouse LDH-5. The serum activity of mouse LDH-5 and of LDH did not correlate with tumor weight.

The presence of mouse LDH-5 in tumor tissue may reflect an ingrowth of mouse cells. In another study the ingrowth of mouse cells was suggested due to the results from karyotyping of cells cultured from an embryonal carcinoma transplanted to athymic mice (Tveit et al. 1980).

The histological and ultrastructural patterns, and the growth pattern of the passages of the tumor in this study were similar to the findings in previous passages (von Eyben et al. 1982).

The high activity of LDH-1 in our testicular germ cell tumor may occur in a large proportion of testicular germ cell tumors with various histological patterns (Zondag 1964; Takeuchi et al. 1979; Andrews et al. 1980). The isoenzyme pattern with predominant LDH-4 and LDH-5 (Akhverdova and Nanobashvili 1977) has not been confirmed in other studies so far.

Studies of testicular germ cell tumor xenografts in athymic mice have several advantages compared with clinical studies and studies of cell cultures. Tumor transplants in athymic mice form a larger part of the total body weight than tumors in men do. In vivo investigations of tumors may be repeated by serial transplantations of the tumors in athymic mice. Histological, ultrastructural, and biochemical studies of the tumors, as well as studies of the tumor growth may be carried out.

The testicular germ cell tumor xenograft in athymic mice is valuable for further studies on the relationship between tumor and LDH and LDH isoenzymes.

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